

A STUDY ON THE PREVALENCE OF OROPHARYNGEAL
CANDIDIASIS IN THE IMMUNOCOMPROMISED INDIVIDUALS
AND THEIR ANTIFUNGAL SUSCEPTIBILITY PATTERN IN
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CONTENTS

Sl.No.	Topics	Page No.
I	Introduction	
II	Review of Literature	
III	Aims & Objectives	
IV	Materials and Methods	
V	Results	
VI	Discussion	
VII	Summary	
VIII	Conclusion	
IX	Suggestion	
	References	

INTRODUCTION

Historically, the fungi were regarded as relatively insignificant causes of infection and only until recently did clinical laboratories offer little more than passing interest in providing mycologic services. It is now well documented that the fungi are common causes of infection, particularly in immunocompromised patients.

The incidence of fungal infections is increasing at an alarming rate, presenting an enormous challenge to healthcare professionals. This increase is directly related to the growing population of immunocompromised individuals, resulting from changes in medical practice such as the use of intensive chemotherapy, radiotherapy and immunosuppressive drugs. HIV and other diseases which cause immunosuppression have also contributed to this problem.

Systemic fungal infections may be caused by either an opportunistic organism that infects an at-risk host, or may be associated with a more invasive organism that is endemic to a specific geographical area. Systemic infections can be life threatening and are associated with high morbidity and mortality. Because diagnosis is difficult and the causative agent is often confirmed only at autopsy, the exact incidence of systemic infections is difficult to determine. The most frequently encountered pathogens are *Candida albicans* and

Aspergillus spp. but other fungi such as non-*albicans Candida* spp. are increasingly important.

OVER VIEW OF CLINICAL MYCOLOGY

More than 50,000 valid species of fungi exist. But only 100 or 150 species are generally recognized as a cause of disease in human beings. Human beings become an accidental host by inhalation of spores or by their introduction into tissue by trauma. Except for the dimorphic fungi, humans are relatively resistant to infection caused by the fungi.

The major factor responsible for the increase in the number of fungal infections has been an alteration in the immune system. Whether caused by the introduction of immunosuppressive agents or serious underlying diseases, this alteration may lead to infection by organisms that are normally considered to be non pathogenic. Such infections occur in patients with

- debilitating diseases such as diabetes
- infection with HIV
- impaired immunologic function resulting from corticosteroid therapy and cytotoxic chemotherapy.
- long term antimicrobial chemotherapy
- long term intravenous cannulation
- gastro intestinal surgical procedures

- malignancies
- organ transplantation

It is because of the need to provide effective health care to immuno compromised that laboratories now must be able to identify and report all fungi from clinical specimen.

GENERAL FEATURES OF FUNGI

Fungi can generally be classified into three groups based on the appearance of the colonies formed.

Yeast & yeast like fungi produce moist, creamy, opaque or pasty colonies on media.

Moulds or filamentous fungi produce fluffy, cottony, woolly or powdery colonies.

Dimorphic fungi exhibit either yeast or yeast like (35°C – 37°C) and filamentous form (25°C – 30°C).

Several pathogenic fungi are dimorphic fungus. Some of the medically important yeasts, particularly the *Candida* spp. may produce yeast forms, pseudohyphae and / or true hyphae. The polymorphic feature of this group of organisms is not temperature dependent.

Yeasts

Yeasts are unicellular organisms that are round to oval and range in size from 2-60µm. *Candida* are oval yeasts 2-6µm×3-9µm in size. The microscopic morphological features usually appear similar for different genera

and are not particularly helpful in their separation. However, the size, presence or absence of a **capsule**, or **narrow necked budding** are features that can be helpful in separation of *Cryptococcus* species from *Candida* species.

Most of the important yeasts and yeast like organisms may belong to the Ascomycota, the Basidiomycota or the Deuteromycota.

In general the yeasts reproduce asexually by blastoconidia formation (budding) and sexually by the production of ascospores or basidiospores. The process of budding begins with a weakening and subsequent **out pouching** of the yeast cell wall. This process continues until the daughter cell (bud) is completely formed. The daughter cell usually detaches from the mother cell by a septum and a residual defect occurs at the site of budding; this is known as a **bud scar**

Alternatively, if the out pouching is tubular without the constriction at its base, a **germ tube** is formed, the initial stage of true hyphae formation. Pseudohyphae represent elongated buds that have failed to separate and are connected to one another to create a “**links of sausage**” appearance. Pseudohyphae will have cell wall constriction rather than true intracellular septations, delineating the fungal cell borders.

TAXONOMY

Berkhout structured the genus *Candida* in 1923 to separate the fungi then known as *Monilia* species into two groups. Plants associated molds still

classified in genus *Monilia*, and yeast associated with warm blooded animals reclassified as *Candida* species.

The genus name *Candida* was formally given the status of nomen conservandum at the International Botanical Congress in Montreal in 1959. Under its current definition, yeasts of basidiomycetous affinity have been excluded from the genus, so that all *Candida* spp. are now yeasts of **ascomycetous** affinity.⁷

Taxonomic revisions lead to flow of additions and subtractions of species name from the list of the “**medical Candidas**”. Several yeasts that earlier enjoyed separate species status are now recognized as synonyms of *C.albicans*, viz. *C.stellatoidea*, *C.clausenii* and *C.langeronii*.

In 1995 a new species, ***C.dublinsiensis*** was defined from Dublin, Ireland. It differs from *C.albicans* in the non-reactivity of its DNA with a *C.albicans* specific molecular probe.¹⁵⁶ Morphologically and physiologically the phenotype of *C.dublinsiensis* closely resembles that of *C.albicans* differing only in minor properties. This new species would never be recognized in laboratories that depend on germ tube test to differentiate *C.albicans* from other yeasts, since germ tube formed by *C.dublinsiensis* is indistinguishable from those of *C.albicans*. *C.dublinsiensis* colonies have a **darker green hue** than those of *C.albicans* on a commercial differential isolation medium.¹⁴⁴ But the presence of intracellular **β glucosidase activity** in *C.dublinsiensis* is the only phenotypic difference that is found with high consistency.

CANDIDA SPECIES - THE PATHOGEN

Several *Candida* species most notably *C.albicans* is an ubiquitous human commensal, residing in the GIT or they are found in the everyday human environment. They become pathogens in situations in which the host's resistance to infection is lowered locally or systemically. In such circumstances *Candida* spp. are capable of causing disease in virtually every location of human body.

Candida infections are commonly encountered in medical practice and changes in the way in which patients become susceptible to *Candida* (eg.more therapeutic immunosuppressive treatment, spread of HIV infection) have led to changes in the epidemiology and clinical presentation of the infection and to a steady rise in their incidence over the last 40 years.

Seven species in the genus *Candida* are well known opportunistic human pathogens and many others have been described as pathogens in individual case reports or short case series.⁵⁸

Species commonly implicated in human infections are,

- **C.albicans**
- **C.glabrata**
- **C.guilliermondii**
- C.krusei**
- **C.lusitaniae**
- **C.parapsilosis**
- **C.tropicalis.**

EPIDEMIOLOGY AND PATHOGENESIS

C.albicans, the species most often associated with human disease has been recovered from many diverse sources apart from vertebrates including the atmosphere, fresh water, seawater and soil. It is an occasional contaminant of foodstuffs and can be recovered from fomites, particularly items that have contacted humans directly such as clothing, bedding and tooth brushes.

Estimates of the prevalence of *Candida* spp. as human commensals vary considerably according to the nature of the site, the type of person sampled and the method of sampling adopted. Many studies of *Candida* carriage in healthy subjects indicate an average prevalence of 25-50% in the mouth,⁹⁶ with *C.albicans*- the predominant species found in 70-80% of cases. In hospitalized patients the oral carriage rates are higher, with yeast-positive cultures obtainable from 50% - 70% of subjects sampled. The prevalence of yeasts detectable in culture is generally lower in other common clinical samples such as feces and vaginal swabs.

It seems likely that virtually 100% of humans may carry one or more *Candida* spp. in deeper intestinal sites (from duodenum to the colon) and that the number of yeasts carried at any point in the GIT often increases to levels that become detectable in the mouth and feces in illness or other circumstances in which the host's microbial suppression mechanisms are compromised.

The half life of *Candida* spp. on the skin of hands has been experimentally determined as a matter of only minutes. However the capability of the yeasts to pass from hand to hand and to inanimate objects has been confirmed,¹¹⁶ and the extended survival of the yeasts on inanimate objects in these experiments tend to confirm the presumption that *Candida* spp. are transmitted to inanimate sources from humans and animals.

VIRULENCE FACTORS

The state of the host is of primary importance in determining *Candida* pathogenicity. Infact, *Candida* spp. are considered opportunistic pathogens because they are usually benign colonizers of mucosal surfaces and because there must be a breakdown of mucosal surfaces or in the host defense for diseases to occur. However, there are factors associated with the organism rather than the host that contribute to its ability to cause disease and explained the differences among species in their pathogenicity.

Adherence of *Candida* spp. to a wide range of tissue types and inanimate surfaces is essential in the early stages of colonization and tissue invasion. Germinated *C.albicans* cells adhere to host tissue more readily than do yeast phase cells.³⁶

Although the data on the yeast hyphal dimorphism status of *Candida* spp. is still inconclusive, **dimorphism** may have some role as a virulence factor. Some information suggests that dimorphism is important (but not essential) in causing disease.¹⁴⁸ Hyphae of *C.albicans* have a sense of touch so

that they grow along grooves and through pores (**thigmotropism**). This may aid infiltration of epithelial surfaces during tissue invasion.

Davis and Denning³⁰ have shown hyphae longer than 200 µm are poorly phagocytosed and not readily killed by leukocytes.

The **hydrophobicity** of the cell surface of *C.albicans* plays an important role in the adhesion of organism to eukaryotic cells and inert surfaces. Blastoconidia are hydrophilic, but germ tube formation is associated with significant rise in cell surface hydrophobicity.¹³¹

The **mannans** also contribute to the virulence of *C.albicans* mainly by two mechanisms. They affect yeast cell surface hydrophobicity and suppress the immune response by mechanisms not yet completely understood.⁹²

Enzyme production is also an important virulence factor. The **aspartyl proteinases** produce non specific proteolysis of host protein involved in defenses against infection allowing entry of yeast beyond connective tissue barriers (*C.albicans*, *C.dubliniensis*, *C.guilliermondii*, *C.parapsilosis* and *C.tropicalis*).¹⁴⁵

Phospholipases are produced by *C.albicans* and in less quantity by non *albicans* spp.(*C.dubliniensis*, *C.glabrata*, *C.krusei*, *C.lusitaniae*, *C.parapsilosis* and *C.tropicalis*).

The **phenotype switching** phenomenon (the formation of differently appearing colony types, particularly those showing discontinuous fringe formation, when cultured on specific synthetic media) may be associated with

the relative virulence of the species.¹⁹⁷It contribute to virulence of *C.albicans* by facilitating its ability to survive, invade tissues and escape from host defenses.

On the other end, neutrophils themselves can augment the switching process towards more susceptible strains.

Another potential virulence factor that is still under study is the **resistance to thrombin - induced platelet microbicidal protein.**¹⁷²

Resistance to azoles also contributes to virulence of the organism.

HOST FACTORS

Local tissue damage

Because *C.albicans* is a culturally weak pathogen, any form of local tissue damage is likely to be important in the pathogenesis of candidiasis. Experimental removal of stratum corneum facilitates the establishment of cutaneous candidiasis.⁸³On the skin, **maceration** is of fundamental importance and in experimental candidiasis; **high moisture** levels usually provided by occlusion are a prerequisite.³²

Iron

In the anemia of leukemia and chronic infections, levels of both **iron** and **unsaturated transferrin** are low, explaining the susceptibility to candidiasis in these groups. **Higgs & Wells**⁶³ showed that some cases of chronic mucocutaneous candidiasis had iron deficiency. With iron replacement therapy their resistance to *Candida* infection increased.

Endocrine factors

In diabetes and Cushing syndrome, the mechanism seems to be a direct suppression of immune mechanism, especially T cell function. In addition, Addison's disease, hypoparathyroidism and hypothyroidism have been found to occur in association with some cases of chronic mucocutaneous candidiasis, in the so-called **Candida endocrinopathy** syndrome.

In many of these patients definite abnormalities of immune factors⁷⁶ are demonstrable which alone are sufficient to explain the increased susceptibility.

Immunological Factors

In the defence against *Candida* infection, both superficial and deep seated, cell mediated immunity (CMI) is of paramount importance coupled with **normal phagocytosis** and killing by polymorphs & macrophages.⁷⁷

Circulating humoral antibodies may have some role⁵¹ and the role of candidiasis is higher in those with **low salivary IgA levels**.²⁵ While systemic corticosteroids act to increase the susceptibility to candidiasis by diminishing immune functions, there is evidence that an inflammatory response to *Candida* can be suppressed by **topical steroids**.¹²² The susceptibility of the elderly and of the seriously ill, especially those with leukemia, reticulosis, carcinomatosis and HIV infection probably lies in large measure, in the depression of CMI.

Racial Factor

The severity of inflammatory response in the American Negroes is

significantly less than that of the American white.¹²² This may be interpreted as showing increased resistance in the black skin.

ROUTES OF TRANSMISSION OF CANDIDA SPECIES

Endogenous transmission

The predominant source of infection in all types of diseases caused by *Candida* spp. is the patient himself or herself. The necessary requirement for invasive disease is a **lowering of a host antiCandida barrier**. Transmission from GIT to the blood stream requires prior overgrowth of the number of yeast in their commensal habitat⁷³ and is favoured by **loss of integrity** of GIT mucosa.¹⁰⁰

Exogenous transmission

Out breaks of *Candida* spp. infection resulting from contaminated materials have been described. Such routes seem to be of significance only in specialized, relatively closed settings such as **burns, geriatric, hematology, intensive care and transplantation units** and also the **newborns**. The hands of hospital personnel may be a potential reservoir. Carriage rate is as high as 58%.¹⁵⁴

DISEASES SPECTRUM

The forms of disease most commonly caused by *Candida* spp. involve the female genitalia, the skin and nails, and the oral cavity, sometimes with concomitant esophageal invasion. *Candida* infections of deep tissues are almost always, the result of hematogenous spread of *Candida* organism from an endogenous or less often an exogenous site. In immunosuppressed patients, particularly with **severe neutropenia** candidemia can result in widely disseminated disease usually with a fatal outcome if untreated.

REVIEW OF LITERATURE

DEFINITION

Candidiasis is a primary or secondary infection involving a member of the genus *Candida*. Because of its rapid ability to colonize and take advantage of many types of host alterations, the clinical manifestations of *candida* infection are protean, ranging from **acute, subacute and chronic to episodic**.

(Rippon JW: Medical Mycology 3rd edition pg.486)¹³⁴

Superficial infections of the mucous membranes and skin are numerically most important but more serious involvement of internal organs as in septicemia, endocarditis and meningitis can also occur. **(Text Book of Dermatology- Rook 4th Edition, pg 946)¹²⁷**

HISTORY

The clinical manifestations of candidiasis have been recognized since ancient times and were discussed in the writings of Hippocrates,⁶⁴ Galen⁴⁸ and Pepy's.¹⁶⁵ In 1839, Langenbeck⁷⁴ described the organism in a lesion of thrush.

Berg in 1841¹³ and Bennet¹⁴ in 1844 conclusively demonstrated the fungal biology of thrush.

Until the genus *Candida* was defined by Berkhout in 1923, a confusing taxonomy ensued. Initially classified as *Sporotrichum* by David Gruby,⁵³ it was placed in the genus *Oidium* (egg shaped, oval yeast cell) by Robin¹²⁸ in 1847. Later it was confused with another fungus isolated from rotting vegetation (*Monilia candida*) by Plaut (1887)¹⁰⁹ and was renamed *Monilia albicans* by

Zopf in 1890.¹⁷³ The dimorphic nature of the fungus was noted by Gravit in 1877⁵⁴ & Audrey in 1887.⁹ In 1853 Robin first described systemic candidiasis. Efforts to speciate yeasts placed in this genus were forged by Martin in 1937.

C.albicans is antigenically divided into two groups, A which it shares with *C.tropicalis* and B which is shared by *C.stellatoidea*.¹⁵⁷ Nucleic acid base composition studies (G-C ratios) indicate that *C.albicans*, *C.tropicalis*, *C.clausenii* and *C.stellatoidea* are related.¹⁵² However, by DNA homology studies, *C.albicans* has close relationship to *C.clausenii* and *C.stellatoidea* but not *C.tropicalis*.¹⁵³

The importance of candidiasis as an opportunistic infection was just appreciated in the post antibiotic era of the 1940s when an increase in the number of candidal infection was noted.

CANDIDA ECOLOGY AND THE COMMENSAL / PARASITE ROLE

Candida as normal flora

In a prospective study of skin colonization, **Henney** et al.,⁶¹ isolated 69 species of filamentous fungi over a 175-day period, but 71% were isolated not more than twice. In contrast, many Blastomycetes (yeast, yeast like fungi) constitute a resident population of the normal flora of skin surfaces, buccal mucosa, the intestinal tract and vaginal mucosa. The species involved and the members present vary with the body surfaces but make up a balanced population in a particular ecological niche on the normal healthy individual.

Oral carriage

Candida albicans is a normal inhabitant of the alimentary tract and the mucocutaneous regions. (**Marples, M.J. The Ecology of the Human Skin**)⁸⁴ It is regularly present in small numbers in the mouths of normal healthy adults. Poor oral hygiene or even small amounts of antibiotics promote an increase in the number of organisms, though usually without untoward results.

Odds et al.,⁹⁸ in his review concludes that *C.albicans* carriage is about 10% in the mouth of normal subjects. Surveys of the oral flora of hospital patients always tend to give higher figures. Odds suggests figures of nearly 24% for yeast carriage and just under 15% for *C.albicans* on the basis of published studies.

Cohen et al.,²⁸ studied that in extensive cases the patches may extend into the trachea, upper bronchi and even into the esophagus. Because the mouth and rectum are easily accessible for swabbing, gut carriage of *Candida* is generally considered in these terms, but of course there is considerable evidence that the yeast colonizes the gastrointestinal tract from end to end, the relative densities being partly dependent on the transient times through the gut.

It is clear from the experiments of **Krause** et al.,⁷³ that the swallowing of massive quantity of *Candida* organisms will result in the transfer of these yeasts rapidly through the gut wall into the circulation, presumably via portal vein and the liver.

Sievers I et al.,¹⁴⁷ studied that yeasts isolated from the mouth are predominantly *C.albicans* (75%) followed by *C.tropicalis* (8%) *C.krusei* (3-6%) and *C.glabrata* (2-6%).

Carmo-sousa L. et al.,²³ studied that in swabs from the anorectal area or feces, 50% of the isolates were *C.albicans* and upto 20% were *C.glabrata* and *C.tropicalis*.

Odds FC et al.,⁹⁸ studied that *C.tropicalis* and *C.albicans* are not regularly found on normal skin but can be recovered from the anogenital region, the area around the mouth and the fingers.

Any damage to skin or mucous membrane or environmental change leads to rapid colonization. Endocrine balance, the administration of steroids, and other physiologic factors also influence the rapidity and extent of *C.albicans* colonization.

C.albicans can occasionally be cultured from environment usually in situations where heavily infected subjects, human or animals are located. **Winner H I & Hurley R.** et al.,¹⁶⁶ have isolated *C.albicans* from a nursery where there was an epidemic of oral thrush and **Clayton Y M & Noble W C**²⁷ isolated from hospital bed linen and **Friedrich E & Blashke-Hellmessen**⁴⁵ from the air of dermatology clinics. Normally, however, Candida is not air borne.

C.albicans is frequently isolated from thermal baths,⁴ heavily utilized beaches^{5,19,146} and occasionally from swimming pools. It is sensitive to

chlorination, (10^5 cells inactivated by 4 ppm Cl at 30 min)⁶⁸ however, will die rapidly in sea water.¹⁹ *C.albicans* can survive for fairly long periods in beach sand,⁵ dairy products and other kinds of food¹²⁹ but not in soil, on plants or in the atmosphere.

Candida associated with human disease

Except for neonatal and conjugal infections most cases of candidiasis probably result from infection of the host by his own commensal yeasts. Certainly isolates of *C.albicans* from normal subjects do not differ enzymatically from those isolated from lesions of thrush,^{26,72} though they have recently been said to differ antigenically.

C.albicans appears to be the only member of this genus regularly able to evoke fatal disease in man and animals. (Mourad et al.),^{87,112} In animal experiments some candida species have been shown to be less virulent than *C.albicans*,¹⁶⁶ a finding which confirms well with known clinical experience.

C.stellatoidea and *C.tropicalis*, though less pathogenic than *C.albicans*, are significantly more virulent than other candida spp. Together *C.albicans* and *C.tropicalis* account for about 80% of the species isolated from medical specimens. (Rippon J W Medical Mycology 3rd edition 1988 pg 532)¹³⁶

Stanford et al., (1980)¹⁵¹ studied that of the various species in the genus Candida, *C.albicans* is the most common cause of superficial and systemic candidiasis. Other Candida spp. particularly *C.tropicalis*, *C.parapsilosis*, *C.kefyr*, *C.guilliermondii*, *C.krusei* and *C.glabrata* also account for some

infections. But the severity of the host's debilitation must be greater to allow these less virulent organisms to invade.

Wingard et al.,¹⁷⁰ in a review of 1591 cases of candidal infections published in 37 reports between 1952 and 1992, found that non- *albicans* spp. were the causative agents in 46% of systemic infections. *C.tropicalis* accounted for 25% of the infections, *C.glabrata* for 8%, *C.parapsilosis* for 7%, and *C.krusei* for 4%. From these reports, patients with malignancies (leukemias) were more likely to be infected with *C.albicans* or *C.tropicalis*.

Hazen⁵⁷ in his extensive review of new and emerging yeast pathogens states that there is increase in non-*albicans* spp., *C.parapsilosis* and *C.glabrata* (bloodcultures), *C.krusei*, *C.guilliermondii*, *C.lipolytica*, and *C.kefyr*.

[Redding SW](#) et al.,¹²³ observed that particularly *C.glabrata*, has emerged as causative agent of oropharyngeal candidiasis (OPC) among immunocompromised patients.

C.dubliniensis is a recently described species that has been shown to cause oropharyngeal candidiasis in patients with HIV. In addition to being a significant cause of candidiasis, *C.dubliniensis* was found to be easily induced to develop resistance to fluconazole in vitro, a phenomenon which may have resulted in its emergence. *C.dubliniensis* produce higher levels of proteinase, are more adherent to buccal epithelial cells and the oral anaerobic bacterium *Fusobacterium nucleatum*, and reveal major ultra structural differences in the cell walls than *C.albicans*. These factors play an important role in the

colonization ability of *C.dubliniensis* in the oral cavity. [Redding SW](#), [Bailey CW](#) et al.,¹²⁴ identified a mixed infection of *C.dubliniensis* and *C.albicans* OPC, in patients undergoing head and neck radiation for oral cancer.

Predisposing factors

Age and sex distribution as well as clinical manifestations of candidiasis are markedly affected by varying predisposing factors and the underlying disease of the patient. Normal equilibrium between Candida and the normal host may be sufficiently upset to lead a pathological state in:

1. Extreme youth: During the normal process of establishing a resident flora, the restricting factors for Candida may be absent, and a clinical condition is produced.
2. Physiologic changes: pregnancy & diabetes(increased carbohydrate content).
3. Prolonged administration of antibiotics.
4. General disability and the constitutionally inadequate patient:

Debility includes slight avitaminosis of the aged, diabetes and its cutaneous candidiasis, candidal vegetations of diseased heart valves, pulmonary or generalized systemic candidiasis as sequelae to chronic disease or as a terminal event in the various neoplasias.

Constitutionally inadequate patients include those with various immune defects associated with abnormal leukocytic functions.

5. Iatrogenic & barrier break candidiasis:

Iatrogenic can result from a wide variety of insults -indwelling catheters, hyperalimentation, or injection of material into the skin, muscle, or circulatory or central nervous system.

Also accidental barrier breaks such as trauma, burns, or gun or knife wounds.

Ecological factors

The effects of ecological pressures from other organisms are of considerable importance. Both in the gut and on the skin, removal of competitive organisms usually, bacteria lead to an increase in yeast numbers, an important prerequisite for the shift to pathogenic role.

In the newborn, however, before an oral ecology is established, even a few organisms presage clinical thrush. In the new born, incidence of oral candidiasis rates as high as 18 % has been recorded, but the average appears to be 4 %. **(Rippon pg 491).**¹³⁵

Work on the competition between candida and bacteria in saliva suggests that the crucial factor is the amount of available glucose¹⁰ and that if this is elevated and plentiful, as in diabetes, the bacterial flora will not inhibit the yeast.⁷² Mechanisms other than nutrition depletion may possibly apply.⁶⁹

Host factors

It has long been recognized that the very old, the very young and the very ill are susceptible to oral thrush.¹⁶⁵ Food debris likely to be present in the mouth of the severely ill patient with inadequate oral hygiene should not be

ignored and may be as significant as diabetic saliva. In Sjogrens syndrome, Candida carriage and probable susceptibility to candidiasis are high, a fact attributed to low salivary flow rates.⁸⁵

Candida infection of oropharynx

Oral Candida infections occur predominantly in patient population, who suffer some kind of systemic or local immunosuppression or who are exposed to other factors that favour the overgrowth and invasiveness of this fungus.

Therefore Candida oral infections are mainly seen in immunosuppressed individuals such as,

Newborns with asphyxia⁵⁵

Persons with diabetes¹³⁰

Patients infected with HIV⁷⁵

Patients receiving corticosteroid or cytotoxic chemotherapy particularly for hematologic malignancies.³³

Patients undergoing maxillo facial radiotherapy³⁷

Recipients of organ or stem cell transplantation³³

Persons exposed to prolonged antibiotic^{130,62} or inhaled steroids¹⁵⁹

Those who wear dentures⁶²

Clinical Syndromes of Oral candidiasis

1). Acute pseudomembranous candidiasis –“oral thrush” (Rippon JW, Medical Mycology 3rd edition pg 492)¹³⁷

This is the commonest form of disease produced by over growth (colonization) of *C.albicans*.The characteristic sign of this condition is a sharply defined patch of creamy white to gray pseudomembrane that covers the tongue, soft palate, buccal mucosa, and other oral surfaces. It is closely adherent to the underlying mucosa, and its removal reveals a red, oozing base. They often crumble and have the appearance of milk curds. Extension to the pharynx or the esophagus may occur.

Acute atrophic oral candidiasis⁷⁸ (antibiotic dermatitis)

May occur de novo or after sloughing of the pseudomembrane of the thrush. It is commonly associated with broad spectrum antibiotic therapy and the use of topical, inhaled or systemic corticosteroids.^{106,115}

Chronic atrophic candidiasis^{20,21}(denture stomatitis)

Affects one in four denture wearers normally confined to the upper denture bearing area. More common in females and 60% of those are older than 65 years of age.

Chronic hyperplastic candidiasis²¹

Very persistent firm, irregular, white plaques occur in the mouth commonly on the cheek or the tongue, which cannot be easily removed. Occurs in AIDS and chronic mucocutaneous candidiasis syndrome. Though it may go

on to malignant change,⁴⁰ it may eventually clear with prolonged anticandida therapy.

Median rhomboid glossitis ²⁹

Characterized by diamond shape area on the dorsum of the tongue with loss of papilla. Occurs as an acquired condition.

Angular cheilitis

Soreness of the angles of the mouth extending to the facial skin.

Candida cheilitis

The center of lip is usually involved.

Oral candidiasis in cancer patients

Oropharyngeal candidiasis is a common infection in patients receiving cancer therapies with an incidence of 5%. Thrush can be present in 28% to 38% of cancer patients undergoing therapy with corticosteroids or cytotoxic agents. **Samonis et al., & Yeo E et al.** ^{143,171}

Oral mucosal colonization (up to 93%) and infection (ranging from 17 to 29%) with *Candida* are particularly common in patients receiving radiation therapy for head and neck cancer. Compromised salivary function secondary to destruction of glandular tissue by radiation is thought to be a major factor leading to *Candida* infection. This infection is marked by oral pain and / or burning and can lead to significant patient morbidity. **Spencer W Redding et al.** ¹⁴⁹

When salivary glands are included in the field of radiation, xerostomia occurs. Radiotherapy-induced hyposalivation encourages oral candidal colonization that often leads to oral/pharyngeal candidiasis. 17.4% developed clinical candidiasis during radiotherapy in the study by [Ramirez-Amador V](#) et al.¹¹⁴

Irradiation mucositis is defined as an inflammatory-like process of the oropharyngeal mucosa following therapeutic irradiation of patients who have head and neck cancer. A scoring method based only on local mucositis signs includes white discoloration, erythema, pseudomembranes and ulceration. Mucositis of the oral cavity in 15 irradiated head and neck cancer patients when studied by [Spijkervet FK](#) et al.,¹⁵⁰ displayed an S-curve reflecting a symptomless first irradiation week, followed by a rapid and steady increase of white discoloration, erythema and pseudomembranes during the second and third week. Oral candidiasis, generalized symptoms such as weight loss and the highest mucositis scores were seen after 3 weeks irradiation.

The presence and use of oral prostheses, alcoholism and smoking as risk factors for oral colonization by *Candida* during radiation therapy were observed by [Epstein J B](#) et al.³⁸

[Patterson T F](#) et al,¹⁰¹ suggest a greater likelihood of developing OPC caused by Non-Albicans Yeasts in patients receiving concomitant chemotherapy and external beam radiotherapy(EBRT).

Patients receiving radiation for head and neck cancer evaluated and cultured weekly for candidiasis, showed mixed colonization with *C.albicans* and *C.dubliniensis*, by **BAILEY C W** et al.¹¹⁷

Severe neutropenia is considered the most significant predisposing factor for life threatening candida infections. In a recent study by **Maksymiuk aw** et al., major candida infections occurred in 84% of leukemia patients who had less than 1000 cells/mm³ for 13 days.⁸² In the setting of neutropenia, there is a high risk of oral candidiasis evolving to systemic infections. This may occur in several ways

1. Direct invasion of the oral mucosa through ulcerations.
2. Extension of oropharyngeal infection into the oesophagus producing oesophageal ulceration, fungemia and dissemination.
3. Extension into GIT.
4. Aspiration of oral secretions causing pneumonia.¹⁶

Oral candidiasis and AIDS

Klein RS et al., studied that oral candidiasis is a common early and often initial manifestation of infection with HIV.⁷¹

Unexplained oral candidiasis in previously healthy adults at high risk for AIDS predicts the onset of other serious opportunistic infections or Kaposi's sarcoma. Over 50% of the time the diagnosis of oral candidiasis preceded the features of AIDS by a mean time of 3 months. The CD4/CD8 ratio was

significantly depressed. The incidence of oral candidiasis in persons with AIDS approaches 100% particularly when CD4 counts are below 200/ μ l.⁵²

Mocroft A et al., studied that oesophageal candidiasis accounts for upto 15% of the AIDS defying illnesses.⁸⁸ **Porro G B , Parente F** et al., noted upto 30% of patients with candida oesophagitis did not have oral thrush.¹¹¹ Similarly only 64-88% of patients with thrush have concomitant oesophageal candidiasis.

The wide spread use of the antifungal agent fluconazole for therapy and prophylaxis in HIV infected patients have resulted in fluconazole resistant strains of *C.albicans* was studied by **Rex J H** et al.,¹²¹ The increased frequency of non albicans strains in the oral mucosa especially among patients with late stage AIDS and those who receive radiotherapy for malignancy of head & neck was observed by **Canuto M D M** et al.,²²

Oral candidiasis in diabetics

High glucose level in urine, in saliva, in general tissue fluids and in sweat may make the diabetics more susceptible to candidiasis.⁷² In a group of diabetics with symptoms of oral candidiasis and denture stomatitis, *C.albicans* was identified in 57.1% of cases. In patients with urinary tract infections the presence of *C.albicans* was shown in 67% of cases. [Dorko E](#) et al.³¹

In non-diabetics exposed in their occupations to high carbohydrate levels, (eg.) bakers an increased level of candida infection in the form of chronic paronychia is recognized clinically. It has been suggested that low

squalene levels and possibly reduced bound carbohydrate are factors leading to candidiasis of skin fold.⁴⁹

Among persons with diabetes, local factors such as smoking, alcoholism and the presence of dentures additionally promote candidal colonization of the mouth.¹⁵⁸ The incidence in debilitated elderly patients has been estimated to be 10%.

Disseminated candidiasis

According to **Bodey G P, Fainstein V** et al.,^{16, 18} the overall incidence of candidiasis has increased persistently world wide during the second half of 20th century. This is due to an increase in the large immunocompromised population resulting from their underlying disease, (malignancy, AIDS) or aggressive treatment with potent immunosuppressive drugs and patients who are sustained for long periods by means of intensive, care increasing the size of the population at risk for candidemia.

C.albicans is still the predominant species causing more than 50% of the cases of candida infection and the fourth leading cause of blood stream infection.¹⁰³

Risk factors for dissemination

Gastro intestinal colonization with candida spp. seems to be necessary for infection. These factors contribute to the development of hematogenous candidiasis through one or all of the following mechanisms.

1. Increased colonization by candida spp.

- a. **Endogenous:** prolonged and multiple antibiotic treatment suppress the endogenous microflora⁵⁶ and enhance the overgrowth of endogenous candida spp at mucosal sites, (especially gut)
 - b. **Exogenous:** prolonged hospital stay increases the risk of acquisition of candida strains present in the hospital environment. (eg. contaminated equipment, health care worker's hands and colonized patients)
2. Alterations in the integrity of GI mucosa leading to increased fungal translocation, by direct vascular invasion when there is mucosal trauma secondary to TPN, malnutrition, surgery, chemotherapy induced mucositis,^{15,95} severe burns and graft-versus-host disease increase the risk of candidemia.
 3. Immunosuppression: Local (increased risk of candida species multiplication or translocation or both) or Systemic (increased risk of hematogenous disease)

Morbidity & Mortality

The mortality attributable to hematogenous candidiasis is high.¹⁶³ The crude mortality rate ranges from 26% - 57%.^{46,86,93,94} Therefore the speed with which antifungal treatment is initiated even if treatment is given empirically can be decisive in a patient's survival. Candidemia is also associated with the 30 day prolongation of hospital stay.¹⁶⁴ A recent prospective study found that

candida spp. was the only microorganism that independently influenced the outcome of bloodstream infections being associated with mortality rates higher than those of other pathogens.⁹⁵ The most important prognostic factors for hematogenous candidiasis are old age, poor performance states, (APACHE – acute physiology and chronic health evaluation) presence and persistence of neutropenia and extensive organ involvement with candidiasis

Diagnosis

In addition to clinical evaluation of the patient, direct microscopic examination of the specimen for the presence of yeast and isolation of yeast in culture is needed for definite proof of infection. **(Rippon J W Medical mycology pg 516)**¹³⁸

Direct examination (Grams stain)

In oropharyngeal candidiasis (OPC), diagnosis can be made by observing typical budding yeasts (2-6µm×3-9µm) with hyphae or pseudohyphae (elongated filamentous cells connected in sausage like manner). The mere finding of yeasts in such materials is of no diagnostic importance. However, mycelial form of organisms usually connotes an established colonization of the involved area. **(Rippon. J W. Medical Mycology 3rd edition pg 516)**¹³⁸

Growth on Sabouraud's dextrose agar (SDA)

C.albicans grows readily on bacterial media, but Sabouraud's dextrose agar (pH 5.6) with added antibiotics is usually recommended for isolation.

All clinical specimens should be freshly taken and cultured on suitable media as soon as possible. Cultures are incubated at room temperature (25°C to 27°C) or 35°C and examined periodically for growth of yeast. Whitish, mucoid colonies grow within 2 to 5 days.

The urease test should also be performed from the isolated colonies on SDA to differentiate the yeast *Cryptococcus*.

Rapid identification of *Candida albicans*

As *C.albicans* is the most important yeast in human disease, many procedures have been proposed for its rapid identification. *C.albicans* is differentiated from other non-*albicans* species by the formation of germ tube; the production of chlamydospores on semi starvation medias like Corn meal agar; absence of chlamydospores on Sunflower Seed Husk Agar and development of opalescence within three days on the Tween80 medium.

Germ tube test (Reynolds-Braude phenomenon)

One of the most reliable methods is incubation of the unknown yeast in 0.5 ml of pooled human serum at 37°C for two hours. **(Rippon.JW Medical mycology pg 517)**¹³⁹Filamentous extension from the yeast cell, with no constriction at the point of origin is considered positive. Germ tube represents the true hyphae whereas the pseudohyphae are derived from the budding process of blastoconidia and failure of cells to get separated from the mother cell. *C.albicans*, *C.stellatoidea* and *C.dublinskiensis* produce germ tube.

Morphology on Cornmeal agar-Tween 80 medium

Transition of growth to the mycelial stage in the absence of fermentable carbohydrate is a characteristic of the genus *Candida*. Corn meal agar is an enrichment medium developed by Hazen and Reed for use in cultivation of fungi. On the Corn meal agar with Tween 80, the commonly encountered human pathogenic and saprophytic species produce rather characteristic mycelial forms and structures. Other members of the genus *Candida*, particularly *C.glabrata* do not form mycelium on the corn meal medium. **(Rippon: 518-525)¹⁴⁰**

Walker and Huppert, in 1960, found that the addition of Tween 80 to corn meal agar resulted in rapid and abundant chlamydospore formation. Among the yeasts encountered in humans, generally only *C.albicans* and *C.dubliniensis* produce chlamydospores on semi starvation medium like corn meal agar. Occasional strains of *C.stellatoidea* and *C.tropicalis* produce these conidia. **(Rippon.JW Medical mycology pg 517)¹³⁹**

Sunflower Seed Husk Agar medium (Khan ZU et al.,⁷⁰ & Pinjon E et al.,¹⁰⁷)

The Sunflower (*Helianthus annuus*) Seed Husk agar medium has been developed and evaluated for differentiation of *C.dubliniensis* from *C.albicans* on the basis of colony morphology and chlamydospore production.

C.dubliniensis isolates produce rough colonies with hyphal fringes and abundant chlamydospores whereas *C.albicans* isolates produce smooth colonies with no evidence of chlamydospore production.

Tween 80 opacity test (Rudek W¹³² & Malcolm Slifkin.⁸⁰)

Many of the pathogenic *Candida* spp. secrete lipolytic enzymes such as esterases and phospholipases.⁵⁰ The esterase activities of these yeasts are demonstrated with the application of Tween 80 opacity medium. The hydrolysis of the Tween opacity medium is associated with the lipolytic enzymes produced by the respective *Candida* spp. Liberated fatty acids bind with the calcium incorporated into the medium. This calcium complex is visible as insoluble crystals around the inoculation site.

All strains of *C.albicans* and *C.tropicalis* produce a halo response within 2-3 days of inoculation and negative response for *C.dubliniensis*. The isolates of *C.guilliermondii* and *C.rugosa* yield a halo at 3-9 days-post inoculation. The Tween 80 opacity test permits the clear differentiation of the strains of *C.albicans* from the strains of *C.dubliniensis* within 3 days of incubation on the Tween 80 medium. It appears to be a useful adjunct that complements the standard morphologic and physiological tests that are used to identify various spp. of *Candida*. The test medium is simple, economical and is easy to interpret.

CHROM agar Candida (CAC)

The new CAC medium aids in the identification of the *Candida* spp. The yeast produces enzymes that react with chromogenic substrates in the CAC medium producing colonies of different colours. These enzymes are specific, allowing some yeasts to be identified to the species level by their colour and

colony characteristics. CHROMagarCandida uses a chromogenic β -glucosaminidase substrate, which is metabolized to give green colonies of *C.albicans* (light green), *C.dubliniensis* (dark green), blue green colonies of *C.tropicalis*, and fuzzy rose-colored or white colonies of *C.krusei*. CAC can be used as the sole primary medium for fungal cultures of specimen in which yeasts are the main fungus sought. When compared to other media commonly used CAC is superior in the primary isolation of yeasts and in identifying mixed colonies of yeasts.

CHROMagar Candida (CAC) is increasingly being reported as a medium used to differentiate *C.albicans* from non-*albicans* Candida spp. (NAC). Rapid identification of NAC can assist the clinician in selecting appropriate antifungal therapy. Some reports have proposed that CAC can also reliably identify *C.dubliniensis* and *C.glabrata*. [Hospenthal DR et al.](#)⁶⁶

CHROMagar candida usually allows atleast the effective differentiation of *C.albicans* from other yeasts (*C.tropicalis* and *C.krusei*) based on one or more of its unique enzyme activities. CHROMagar candida is an ideal medium for Candida isolation because it has revealed mixtures of candida spp.in many types of clinical specimens more often than would have been expected. For laboratories unable to use this isolation medium for economic reasons, it is still possible to take advantage of it to look for mixed yeast populations only in positive cultures obtained with a cheaper medium.

CAC was noted to recover more yeast isolates than RM (routine media) when mixed cultures were detected. [Murray CK](#) et al.⁹⁰

Ribot, W R Kirkpatrick et al.,¹²⁶ suggests a higher likelihood of developing OPC in patients receiving concomitant CRT. Chromogenic media is helpful to screen for relatively, commonly occurring non- *albicans* yeasts causing OPC in head & neck cancer patients.

Confirmation of Candida species

Carbohydrate assimilation test

If there is difficulty in deriving a presumptive identification from the growth patterns on Cornmeal agar, carbohydrate fermentation or assimilation tests may be required for final species identification. Assimilation test is preferred as the fermentation tests give more false positive results due to endogenous carbohydrates. (**Elmer Koneman, Color Atlas and Text Book of Diagnostic Microbiology, 5th edition, 1043**)³⁴

ANTIFUNGAL SUSCEPTIBILITY

Blood stream infections caused by *C.albicans* and *C.tropicalis* are more likely to occur among patients who do not receive fluconazole therapy.¹The use of azoles in prophylactic antifungal treatments may increase the likelihood of infection by *C.glabrata* and *C.kursei*¹⁶⁸ but doesn't seem to influence the likelihood of infections by *C.parapsilosis*.

Oropharyngeal candidiasis with non-*albicans* spp. *C.glabrata* and *C.krusei* are being isolated with increased frequency in HIV patients and are

commonly less susceptible to fluconazole than *C.albicans*. Also, the presence of non-*albicans* Candida was more frequently associated with severe symptoms. [Redding SW](#) et al.¹¹⁸

C.glabrata has emerged in recent years as a significant cause of systemic fungal infection. Development of resistance to fluconazole by *C.glabrata* is a highly varied process involving multiple molecular mechanisms. [Redding S W](#) , [Kirkpatrick W R](#) et al.¹¹⁹

Of note *C.dubliniensis* is a novel species that may be misdiagnosed as fluconazole resistant *C.albicans*.¹¹³

Prolonged antifungal therapy will induce resistance. Multiple observations have demonstrated that this event is frequent⁸¹ On the other hand, it has also been shown that antifungal agents can reduce the rate of relapse and colonization.¹¹⁰

For this reason, the susceptibilities of isolates recovered from compromised patients should be monitored. In order to do this in a cost-effective way, simple, inexpensive testing procedures are needed, but such tests must be accurate and precise.

Recently the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee on Antifungal Testing has proposed an agar disk diffusion method, M44-P, for testing of fluconazole against yeasts. This method has been shown to be accurate and precise and correlates well with the NCCLS broth microdilution (BMD) MIC method when testing *Candida*. Zone

diameter interpretive criteria have been developed along with reference MIC correlates for the categories of susceptible (S) (19 mm [≤ 8 $\mu\text{g/ml}$]), susceptible—dose dependent (SDD) (15 to 18 mm [16 to 32 $\mu\text{g/ml}$]), and resistant (R) (≤ 14 mm [≥ 64 $\mu\text{g/ml}$])

Aim

To study the prevalence of oropharyngeal candidiasis in the immuno compromised individuals mainly, patients on radiotherapy, HIV infected and the diabetic patients who attended Tirunelveli Medical College Hospital.

Objectives

1. To detect the occurrence of Candida by
 - Grams staining
 - Culture on Sabouraud's Dextrose Agar
2. To speciate the Candida by
 - Germ tube test
 - Growth on Cornmeal Agar Tween 80
 - Sunflower Seed Husk Agar
 - Opalescence on Tween 80 Medium
 - Specific colour on CHROM agar candida
 - Morphology on Cornmeal Agar Tween 80
 - Carbohydrate Assimilation on Yeast nitrogen base medium
3. To find out the antifungal susceptibility of the candida isolates by the disc diffusion method approved by NCCLS M44 – P document.

MATERIALS & METHODS

This prospective study was conducted at Tirunelveli Medical College Hospital for a period of 18 months from January 2005 – June 2006. The study group included immunocompromised individuals – patients who received radiotherapy for malignancies of the oral cavity, patients attending the Voluntary Counseling and Testing Center (VCTC), and the patients attending the diabetic out patient department.

SELECTION OF PATIENTS

Inclusion criteria

1. Patients who had received external beam radiotherapy (EBRT- 45 Gy in 15 days, 3Gy / day) and developed oral candidiasis.
2. Patients who were proved HIV (human immunodeficiency virus) positive by double ELISA method with clinical stages B1, B2, B3 according to CDC classification. (Symptomatic and CD4 count $>500\text{cells}/\mu\text{l}$ (B1), $200\text{-}500\text{cells}/\mu\text{l}$ (B2), $<200\text{cells}/\mu\text{l}$) or stage 3 of WHO clinical staging.
3. Patients with uncontrolled diabetes for more than a year.

Exclusion criteria

1. Patients on EBRT (external beam radiotherapy) for oral malignancies for <15 days without any oral complication.

2. Patients proved HIV positive by double ELISA method but who were not in stage 3 of WHO clinical staging.
3. Patients with known history of diabetes for a short period (6 months).

A total of 190 immunocompromised individuals were studied and evaluated for the occurrence of candidiasis. Speciation & antifungal susceptibility pattern of the yeast isolates were done.

SPECIMEN COLLECTION

History was elicited regarding the risk factors like smoking, alcoholism and tobacco chewing.

The CD4 cell count was noted in all the HIV infected individuals.

Patients were screened for the presence of white patch on the tongue, mucosa of the hard and soft palate, and the buccal cavity. Patients were also screened for glossitis of the tongue.

Two oral swabs were taken from the lesions using sterile Himedia dry cotton swab, one for direct smear and the other for culture.

SPECIMEN PROCESSING

DIRECT EXAMINATION – GRAMS STAIN

Grams stain was performed on all the 190 samples and screened for budding yeast cells by direct examination.

Smears prepared from the oral swab were fixed, stained and examined under oil immersion objectives (100 x).

Presence of gram positive, oval budding yeast cells (2-4µm), elongated filamentous cells, connected in a sausage like manner (pseudohyphae) or as truly septate hyphae were consistent with the morphology of *Candida* spp.

CULTURE METHODS

Species identification relies on isolation of the yeast in pure culture followed by biochemical and physiologic tests.

PRIMARY ISOLATION ON SABOURAUD'S DEXTROSE AGAR (SDA)

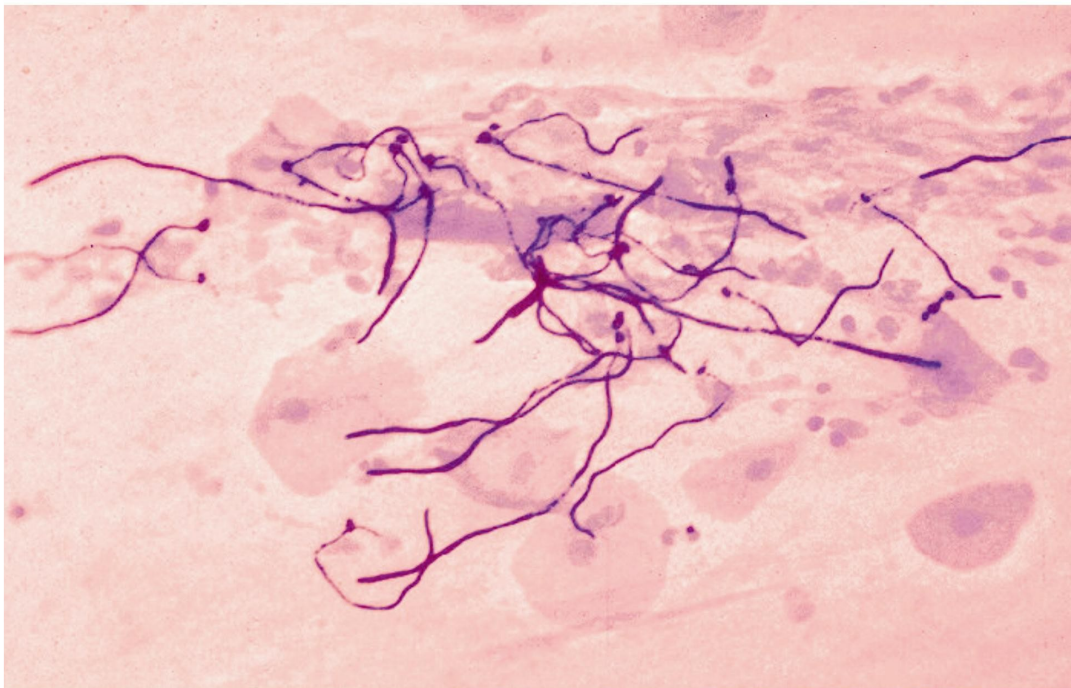
Oral swabs freshly taken using sterile, Himedia dry cotton swab were cultured onto Sabouraud's dextrose agar (pH5.6) containing chloramphenicol (50mg/l) and gentamicin (20mg/l) to minimize bacterial contamination. Cycloheximide was not incorporated in isolation media, because it inhibits the growth of some species (*C.tropicalis*, *C.krusei*, and *C.parapsilosis*). The inoculated SDA plates were incubated at 35°C for 48-72 hrs.¹⁷³ Relatively large, butyrous colonies were easy to distinguish and isolated colonies from each positive culture were selected and again subcultured to SDA slants for stock culture.

UREASE TEST

A portion of the colony from SDA (Sabouraud's dextrose agar) was inoculated to the surface of a Christensen's urea agar slant and incubated at 37°C for 18-24 hours.



Sabouraud's Dextrose Agar



Grams stain

The change in color of the slant to pink is a positive test, differentiating the yeast *Cryptococcus* from *Candida*, which is urease negative. **(Koneman, Color Atlas and Diagnostic Microbiology 5th edition pg 1043)³⁴**

RAPID IDENTIFICATION OF C.ALBICANS

Since *C.albicans* accounts for majority of the *Candida* infections, all isolates were subjected to the following tests:

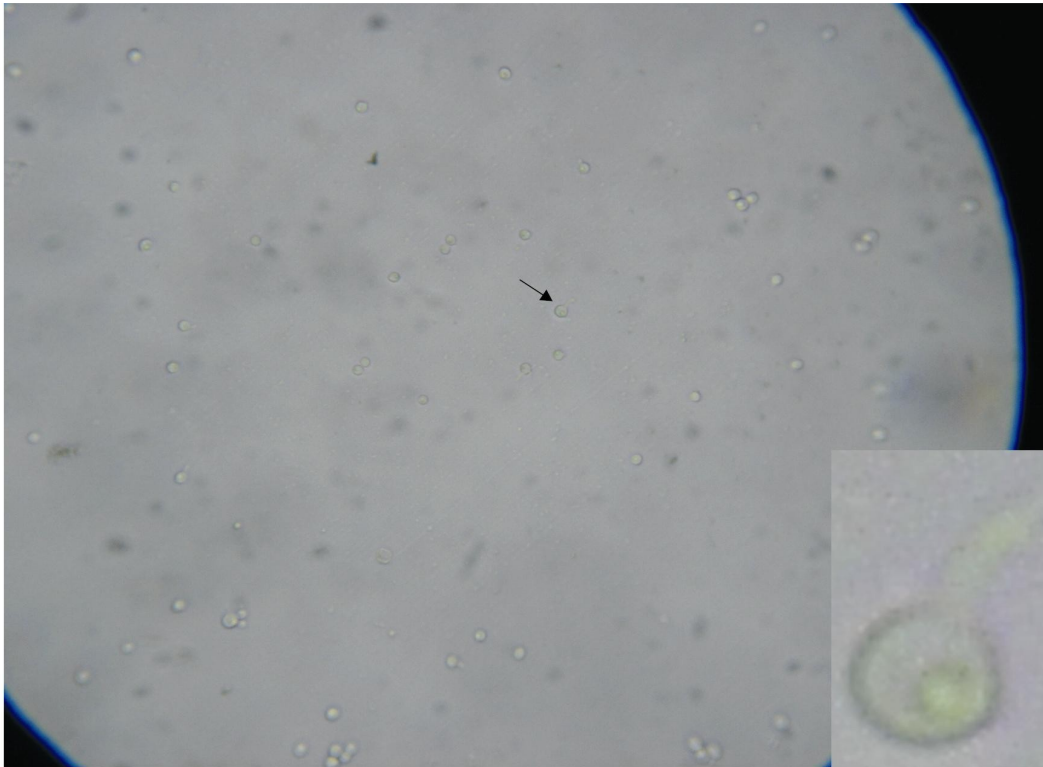
- ❑ **Germ tube test**
- ❑ **Production of chlamyospores on Corn Meal-Tween 80 agar**
- ❑ **Production of chlamyospores on Sunflower seed husk agar**
- ❑ **Tween-80 opacity test**
- ❑ **CHROMagar Candida**

GERM TUBE TEST (REYNOLDS –BRAUDE PHENOMENON)

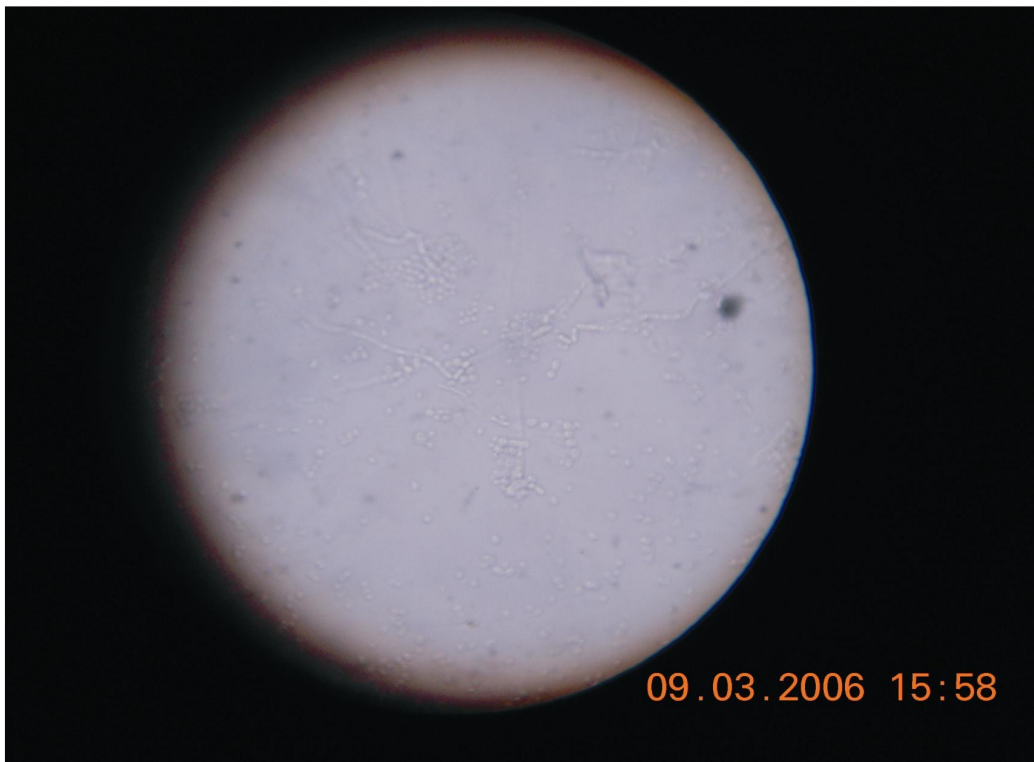
Procedure

A small portion of isolated colony of yeast to be tested was suspended in a sterile test tube containing 0.5 ml of pooled human serum and incubated at 37°C for two hours. A drop of yeast serum suspension was placed on a microscopic slide, overlaid with a coverslip and examined microscopically for the presence of germ tube. The test is not valid if examined after 2 hrs. **(Rippon.JW Medical mycology pg 517)¹³⁹**

Filamentous extension from the yeast cells about ½ the width, and 3-4 times the length of the cell, with no constriction at the point of origin was considered positive. Germ tube represents the true hyphae.



Germ Tube



C.glabrata-CMA T 80 Medium

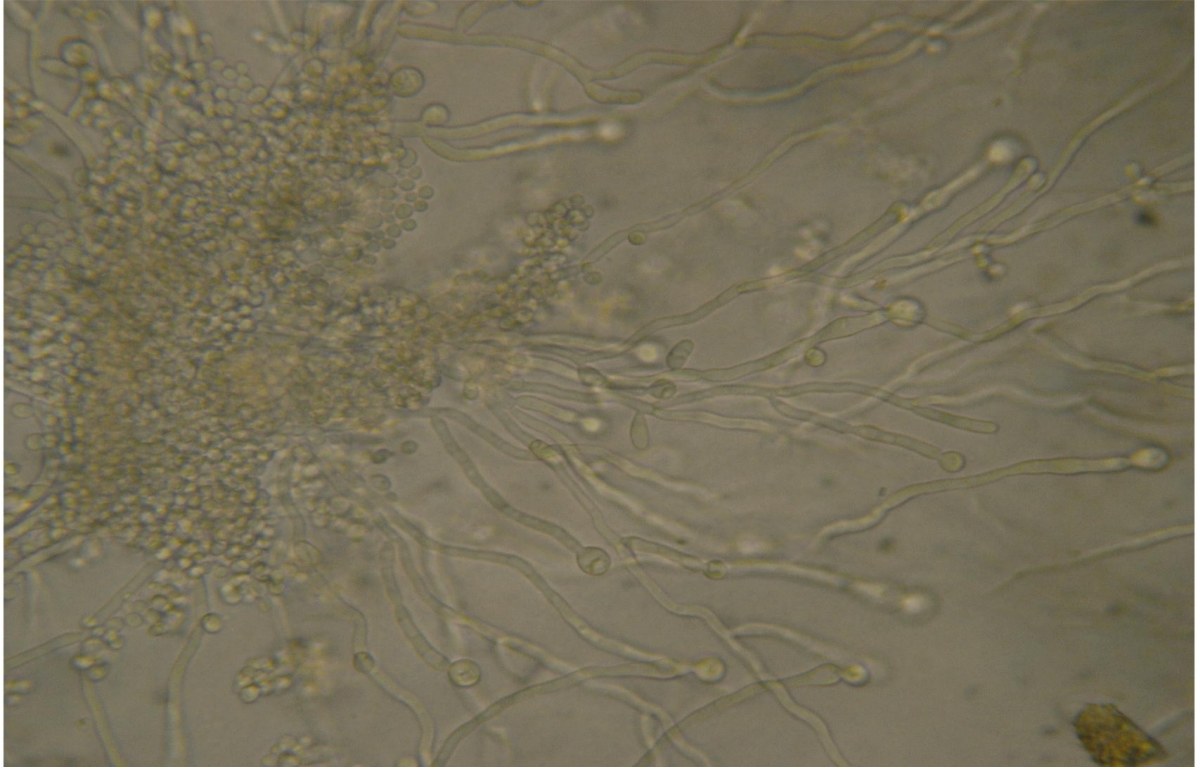
CORMEAL AGAR WITH TWEEN 80

Cornmeal (Himedia) 2gm, agar 15gm and Tween 80 -7ml were added to 1 liter of distilled water, pH adjusted to 6.2 and autoclaved at 121 °C, 15lbs for 15 mins. Medium was cooled to 45-50°C, poured into sterile Petri dishes and allowed to solidify for at least 30 mins.

Tween 80 (polsorbate-0.02%) was added to corn meal agar to reduce the surface tension and enhance the formation of hyphae and blastoconidia. The morphology of the blastoconidia, chlamydospores and the ability to produce pseudophyphae helped in the identification of the species. **(Rippon.JW Medical mycology pg 517)**¹³⁹

Procedure:

1. Using an inoculating needle, a visible paste of the organism was obtained. The needle was drawn through the agar making two perpendicular lines in the shape of an 'x'.
2. A cover slip was flamed, allowed to cool and placed over the central area of the 'x' in order to reduce the O₂ tension. Reduced O₂ tension stimulated the chlamydospore production.
3. The plate was sealed with a tape and incubated aerobically at 25-30 °C for up to 72 hours.
4. Inverted plates were examined microscopically using a low power objective, along the edge of the coverslip for detection of



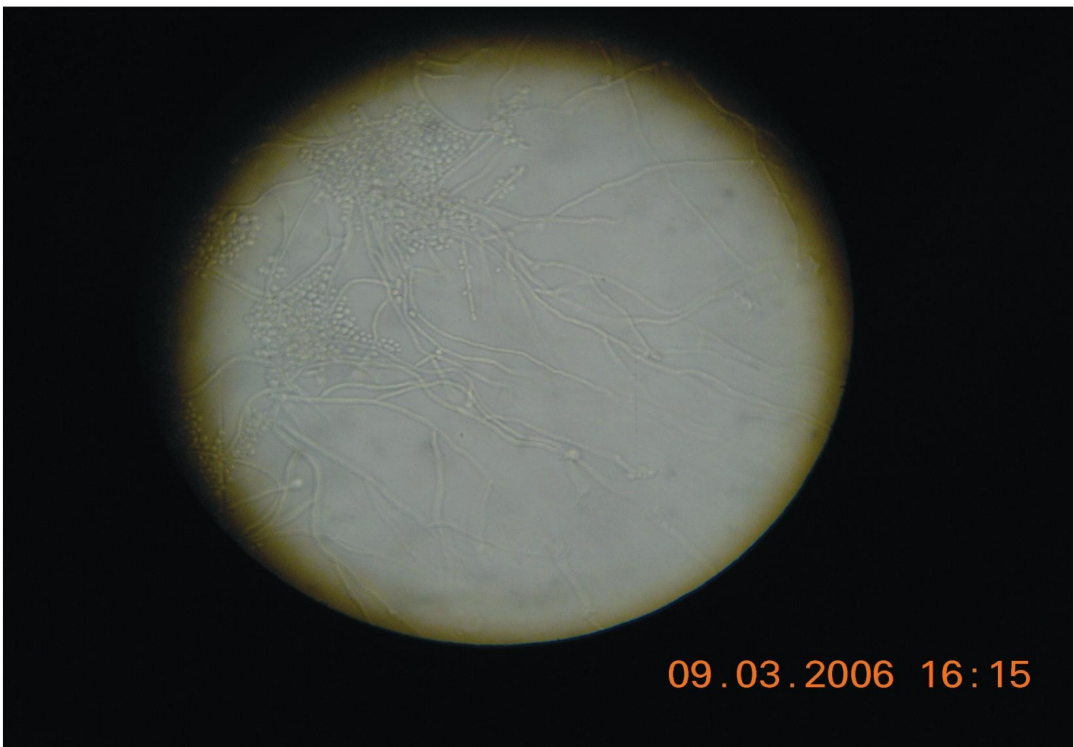
Chlamydospores on CMA T-80 *C. albicans*



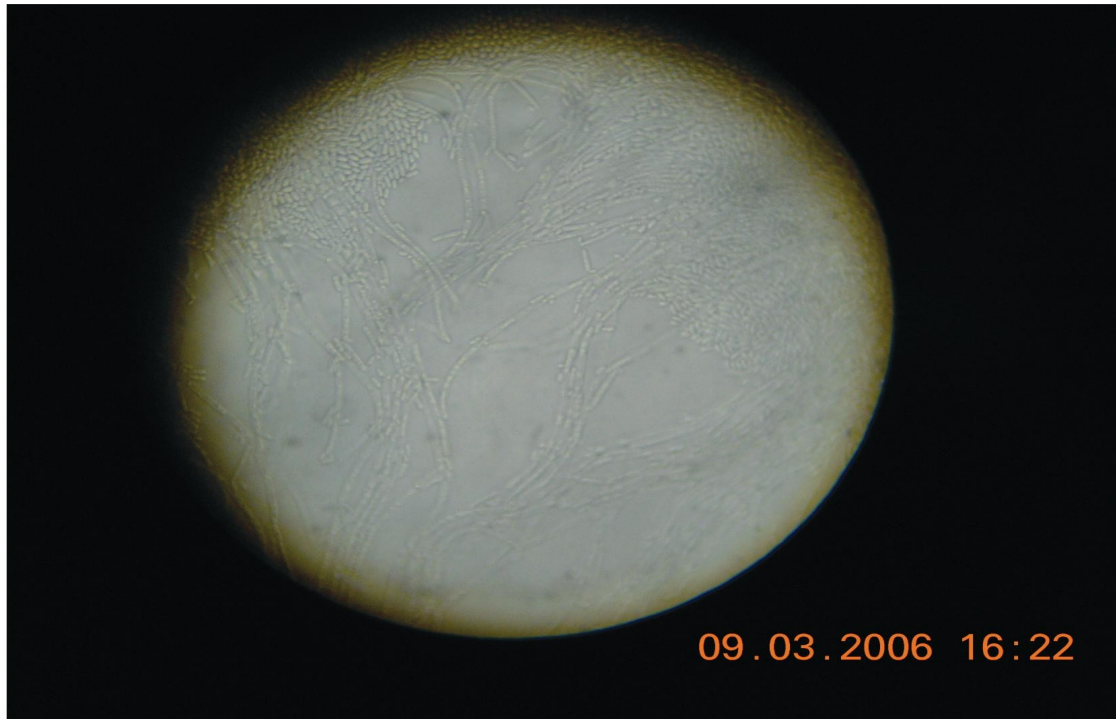
Chlamydospores on CMA T-80 *C. dubliniensis*



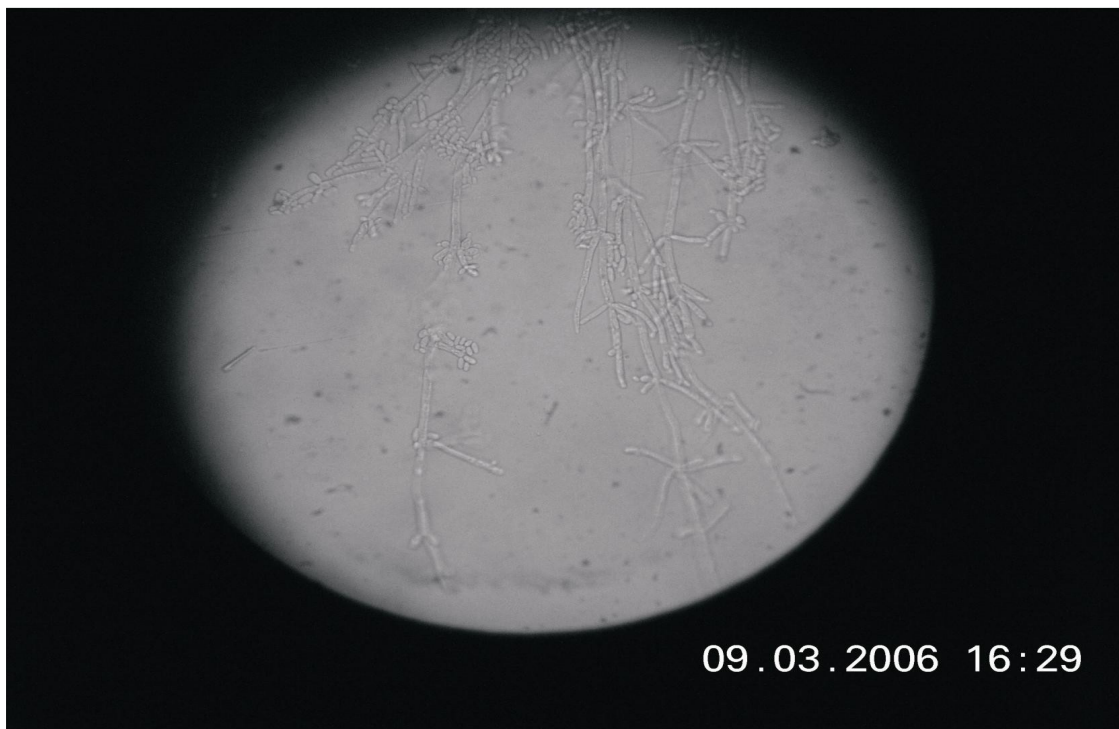
Corn meal Tween-80 *C. tropicalis*



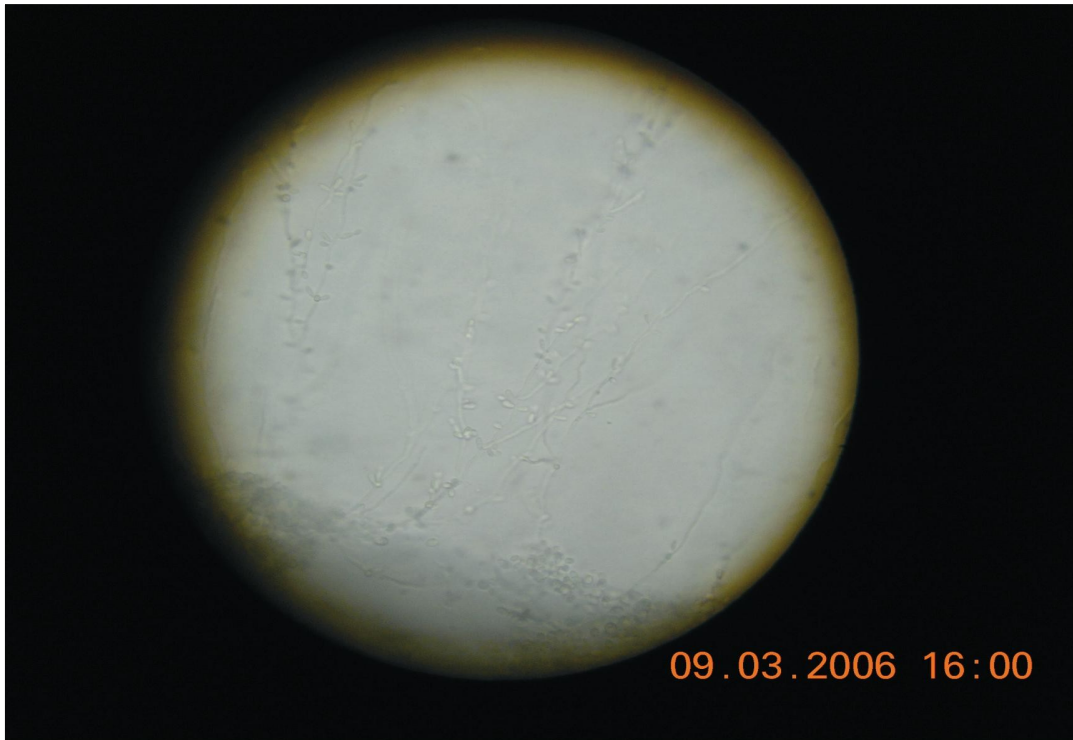
Corn meal Tween-80 *C. parapsilosis*



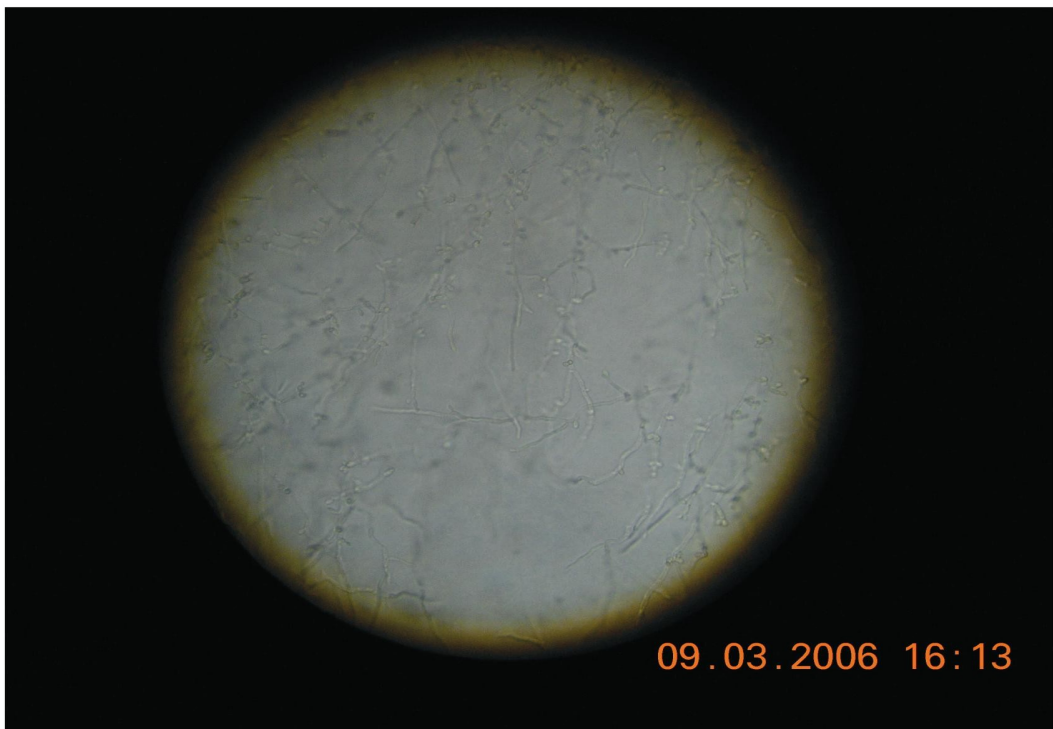
Corn meal Tween-80 *C. kefyr*



Corn meal Tween-80 *C. krusei*



Corn meal Tween-80 *C. guilliermodii*



Corn meal Tween-80 *C. lusitaniae*

chlamydospores.(Koneman, Color Atlas and Diagnostic Microbiology 5th edition pg 1043 & Hardy and Hugo diagnostics)³⁴

Interpretation

C. albicans: produce compact clusters of blastoconidia at regular intervals along pseudohyphae. (Intercalary or terminal chlamydospores).

C.dubliniensis: produce clusters of chlamydospores usually in doublets or triplets.

C.tropicalis produce abundant pseudomycelium composed of elongated blastoconidia widely spaced, in single or small clusters along the hyphae.

C. parapsilosis produce thin, much branching pseudomycelium and verticals of few ovoid to elongate blastoconidia. Thick pseudomycelium and giant cells may be present.

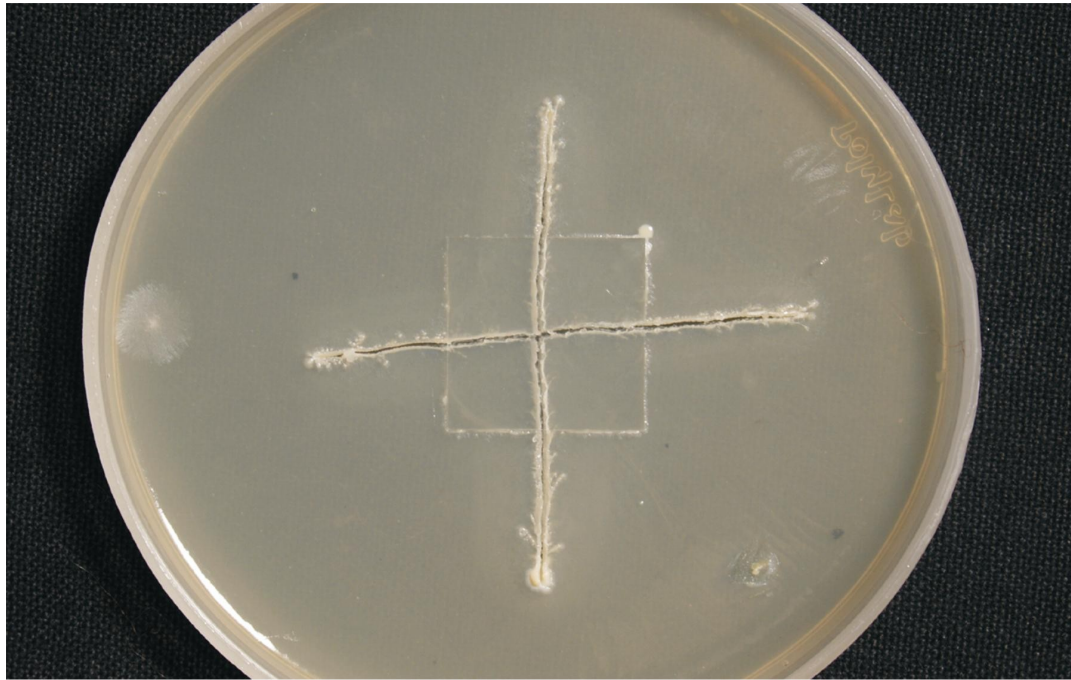
C.krusei produce elongated blastoconidia in tree or crossed match stick like arrangements.

C.glabrata produce no pseudomycelium, but only small round yeast cells are seen.

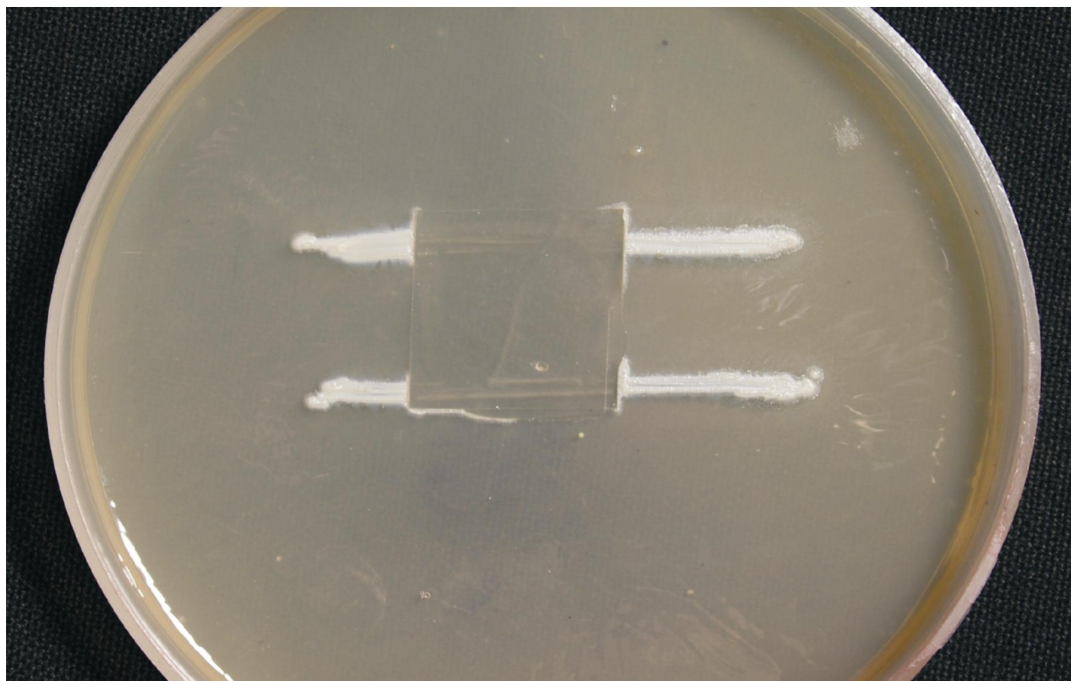
C.kefyr produce abundant pseudo mycelium with very elongated blastoconidia, lying parallel like logs in a stream. Blastoconidia are not abundant.

SUNFLOWER SEED HUSK AGAR

As per **Khan ZU** et al.,⁷⁰ & **Pinjon E**, et al.,¹⁰⁷ sunflower (*Helianthus annuus*) seed husk agar medium was prepared and evaluated for



Corn Meal Agar-Tween 80 Medium



Sunflower Seed Husk Agar Medium

differentiation of *C.dubliniensis* from *C.albicans* on the basis of **colony morphology** and **chlamydospore** production.

The sunflower seeds were purchased from open market and the husk was separated manually. The medium was prepared as follows: 50gram sunflower seed husk was pulverized in domestic grinder for 3-4 minutes. The pulverized husk was boiled for 30 minutes with 1 liter of distilled water and filtered through several layers of gauze. To the husk extract so obtained were added 15gram agar and 10 gram glucose. The volume was made up to 1 liter and pH adjusted to 5.5 before autoclaving at 121°C, 15 lbs for 15 minutes.

Dalmau plate culture

All the isolates forming germ tubes and chlamydospores on Corn Meal Agar–Tween 80 (CMA-T80) were streaked on Sunflower Seed Husk (SSHA) Agar for studying the colony morphology and chlamydospore production. With a sterile inoculating needle, two streaks, approximately 1.5 cm long, were made on the media one centimeter apart without digging into the medium. Only a very small quantity of the culture was streaked. After flaming and cooling the needle, an S-shaped streak was made across the two streaks made earlier. Coverslip (22 × 22 mm) was flame sterilized, cooled and placed over the streak marks. The plates were incubated at 28°C and examined under microscope using low (10x) and high power (40 x) at 24, 48 and 72 h intervals.

C.dubliniensis isolates produce rough colonies with hyphal fringes and abundant chlamydospores whereas *C.albicans* isolates produce smooth colonies with no evidence of chlamydospore production.

TWEEN 80 OPACITY TEST

Plain agar medium was prepared with 10gm peptone, 5gm NaCl, 0.1gm CaCl₂, 15gm agar, and 1000ml distilled water. After the medium was autoclaved at 121°C, 15lbs for 15 mins. It was cooled to about 50°C, and 5ml of autoclaved Tween 80 was added. The final pH of the medium was adjusted to 6.8. The 90 mm petri dishes were filled with 25ml of the medium.

Overnight cultures of each isolate grown on SDA were transferred to the Tween 80 medium by touching the center of agar medium with a sterile cotton swab to prepare a circular inoculation site about 10mm in diameter. The inoculated agar plates were incubated at 30°C and were examined daily for 10 days.

The presence of a halo around the inoculated site on Tween medium viewed with transmitted light indicated a positive test, that the isolate produced an esterase. The fatty acids liberated on hydrolysis of Tween 80 bind the calcium incorporated in medium to form a visible calcium complex of insoluble crystal. All strains of *C.albicans* and *C.tropicalis* produce a halo response within 2-3 days of inoculation. The isolates of *C.guilliermondii* and *C. rugosa* yield a halo at 3-9 days post inoculation and negative response for *C.dubliniensis*,^{132,80} even after 10 days.

CHROM AGAR CANDIDA:

CHROMagar Candida (CAC) is a selective medium for the isolation of fungi that simultaneously provides direct differentiation and identification of several *Candida* spp.

This medium contains agar 15gm, peptic digest of animal tissue 15gm, dipotassium hydrogen phosphate 1gm, chromogenic substrate 11.22gm and chloramphenicol 0.5gm per liter. 42.72 grams of CHROMagar Candida (Himedia) was suspended in 1000 ml distilled water, and boiled to dissolve the medium completely. The medium was cooled to 50°C and poured into sterile Petri plates. The medium should not be autoclaved.

A suspension of yeast was made from an overnight culture on Sabouraud's dextrose agar and inoculated to CAC. The plates were incubated in atmospheric air at 37°C as recommended by manufacturers. All cultures were read at 48 hrs. Colonies of *C.ablicans* and *C.dubliniensis* appear **lighter** and **darker green**, respectively. *C.tropicalis* appear with a **blue green hue** and *C.krusei* colonies appear **light pink** or **white** and **dry** with a light border. Other yeasts appear cream colored.

CONFIRMATION OF THE CANDIDA SPECIES

Morphology on Cornmeal agar with Tween 80

- ❑ **Sugar Assimilation test**
- ❑ **Tween 80 opacity test**

CHROM agar Candida medium



C. albicans



C. krusei



C. tropicalis



C. kefyr



CHROM agar Candida

MORPHOLOGY ON CORNMEAL AGAR WITH TWEEN 80

The morphology and arrangement of the blastoconidia, pseudohyphae and the hyphal arrangements were observed for speciation of the *Candida* isolated.

CARBOHYDRATE ASSIMILATION TEST

As the fermentation tests give more false positive results due to endogenous carbohydrates, **assimilation test** was performed to assess the ability of the yeasts to grow in various single carbohydrate substrates.

Procedure

Yeast nitrogen-base agar(YNB):

2% agar solution was prepared, autoclaved for 15mins, at 121°C at 15psi. 6.7gm of YNB (Himedia) was dissolved in 100ml of distilled water. The pH was adjusted to 6.2-6.4 by adding 1 N NaOH and filter sterilized. To 1 liter of 2% agar solution, 88ml of YNB and 100ml of filter sterilized bromocresol purple indicator was added.

A standardized suspension of yeast in saline equivalent to McFarland standard 4 was prepared. It was allowed at room temperature, overnight to exhaust their endogenous metabolites. Viability control of this inoculum was accompanied, by inoculating the suspension onto SDA and observed for the growth of the yeast isolate at 37°C for 24- 48 hours. **(Gradwohl's Clinical Laboratory Methods and Diagnosis, 8th edition, Alex. C. Sonnen wirth. Leonardjarett pg. 2036)**³

Carbohydrate Assimilation Test



C. albicans



C. tropicalis

With a sterile Pasteur pipette, the surface of the yeast nitrogenous base agar plate was flooded. Excess suspension was aspirated using the same pipette. The surface of the agar was allowed to dry for 5 minutes.

Glucose, Galactose, Lactose, Maltose, Sucrose, Trehalose, Xylose, Raffinose, Cellobiose and Melibiose carbohydrate assimilation (Himedia) disks were placed on the agar and pressed down firmly with the tip of flamed forceps. The disks were placed sufficiently far apart so that the zone of diffusion from one carbohydrate disk did not overrun the diffusion zone of another (one in each quadrant and one in the center).⁶⁵ The plates were incubated at 30°C for 24 hours.

Result

A positive test was the observation of visible growth around the carbohydrate disk, indicating that the sugar contained has been assimilated by the yeast species under study. The size of zone or the density of growth around the disk is unimportant (**Elmer W. Koneman, Color Atlas & Text book of Diag. Microbiology 5th edition pg 1305**).³⁵

ANTIFUNGAL SUSCEPTIBILITY TESTING (NCCLSM44-P) A. Barry, 1 et.al.¹¹

Media preparation

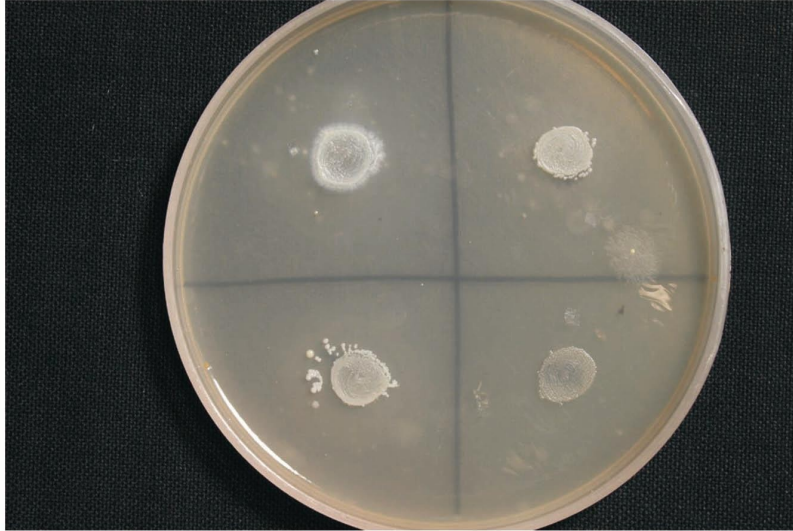
Muller-Hinton (MH) agar was used for the antifungal susceptibility test. A stock solution of methylene blue (5 mg/ml) was prepared and refrigerated at

2 to 8°C. To 100 ml of a 40% glucose solution, 200 µl of the stock methylene blue was added to give 10 mg of methylene blue per ml of 40% glucose to give 10 mg of methylene blue per ml of 40% glucose (GMB solution). The glucose methylene blue (GMB) solution was dispensed into screw-capped tubes as 1.5 ml/tube for 100-mm-diameter MH plates. The solution was then sterilized by autoclaving.

Flooding procedure

The day before testing, refrigerated tubes with the GMB solution were allowed to warm to room temperature and at the same time MH agar plates were dried in a 35°C incubator with lids ajar until all of the surface moisture evaporated for 1 to 2 hrs. The dried agar surface was then flooded with 1.5ml of GMB solution, and the liquid was allowed to adsorb at room temperature on a flat surface overnight. Assuming that the supplements were completely adsorbed and evenly dispersed throughout the agar in each 100-mm-diameter plate, the final concentrations of glucose and methylene blue was 2% and 0.5 mg/ml, respectively.

Disk diffusion tests were performed by preparing a saline suspension of freshly isolated colonies that was then adjusted to match the turbidity of a McFarland 0.5 standard. A sterile applicator swab was moistened in this cell suspension and then used to inoculate the surface of each 150-mm-diameter agar plate. After inoculation of the plates the Fluconazole disk was applied to the surface. Also Nystatin, Clotrimazole, Ketoconazole, Itraconazole and



Tween-80 opacity Test



Antifungal susceptibility Test

Amphotericin B disks were applied to the surface of the agar with a distance of about 24mm for comparison. The inverted plates were then incubated at 35°C for 20 to 24 h. Kirby-Bauer scale were used to measure the diameter of each zone of inhibition at the point where there was a sharp decline in the density of growth.

Result

All strains tested for susceptibility were interpreted as belonging to one of three categories:⁹¹

Susceptible: - strains for which zone diameters were ≥ 19 mm

(Corresponding to MICs ≤ 8 $\mu\text{g/ml}$)

Susceptible-dose dependent:-strains for which zone diameters were 15 to 18 mm

(Corresponding to MICs 16 to 32 $\mu\text{g/ml}$)

Resistant: - strains for which zone diameters were ≤ 14 mm

(Corresponding to MICs were ≥ 64 $\mu\text{g/ml}$)

RESULTS

A total of **190** oropharyngeal (OPC) swabs were collected from the patients of Radiotherapy unit (RT), Voluntary Counseling and Testing Center (VCTC) and Diabetic out patient department of Tirunelveli Medical College Hospital.

In the radiotherapy unit, **90** OPC samples were collected from patients who had received External Beam Radio Therapy (EBRT) for malignancy of the oral cavity for a period of 15 days. In VCTC, **58** samples were taken from HIV infected individuals. In the Diabetic out patient department, **42** samples were taken from individuals who had uncontrolled diabetes mellitus for more than a year. **(Table: 1) (Fig.1)**

Table 1: Department and sex wise distribution of samples

Department	Male	Female	Total
Radiotherapy unit	54 (60)	36(40)	90
VCTC (HIV +ve)	30(51.7)	28(48.3)	58
Diabetology	34(81)	8(19)	42
Total	118 (62.1)	72 (37.9)	190

(Parenthesis denotes percentage)

Of the **190**-study population, **118** (62.1%) were male and **72** (37.9%) were female. Among them **7** (3.7%) were < 20years, **14** (7.4%) were between 21-30 years, **56** (29.5%) were between 31 - 40 years, **48** (25.3%) were between

41-50 years, **33** (17.4%) were between 51-60 years and **32** (16.8%) were >60 years. (Table 2)(Fig.2)

Table 2: Demographic character of the study population

Age	Radiotherapy		VCTC		Diabetology		Total
	M	F	M	F	M	F	
< 20 years	---	---	2	4	---	1	7(3.7%)
21-30	---	---	8	4	1	1	14(7.4%)
31-40	4	8	14	20	5	5	56(29.5%)
41-50	8	20	6	0	13	1	48(25.3%)
51-60	18	6	---	---	9	0	33(17.4%)
>60	24	2	---	---	6	0	32(16.8%)
Total	54	36	30	28	34	8	190

(Parenthesis denotes percentage)

PRIMARY ISOLATION OF CANDIDA SPECIES FROM CLINICAL SAMPLES

Grams staining and growth on Sabouraud's dextrose agar (SDA)

When **190** oropharyngeal (OPC) swabs were examined by Grams staining and cultured on Sabouraud's dextrose agar (SDA), **133** (70%) showed budding yeast cells on direct examination and creamy growth on SDA. In Grams staining **54** (40.6%) showed pseudohyphae.

Out of the **90** samples from the patients who had received radiotherapy, **64** (71.1%) were positive for budding yeast cells and growth on SDA. Of the **58** samples from the HIV infected individuals, **30** (51.7%) were positive for

budding yeast cells and growth on SDA. Of the **42** samples from the diabetics, **39** (92.8%) were positive for budding yeast cells and growth on SDA (**Table 3**) (**Fig.3&3a**).

From the Grams smear and growth on SDA **133** (70%) out of **190** samples were identified as Candida.

Fig. 1. Department and sex wise distribution of samples

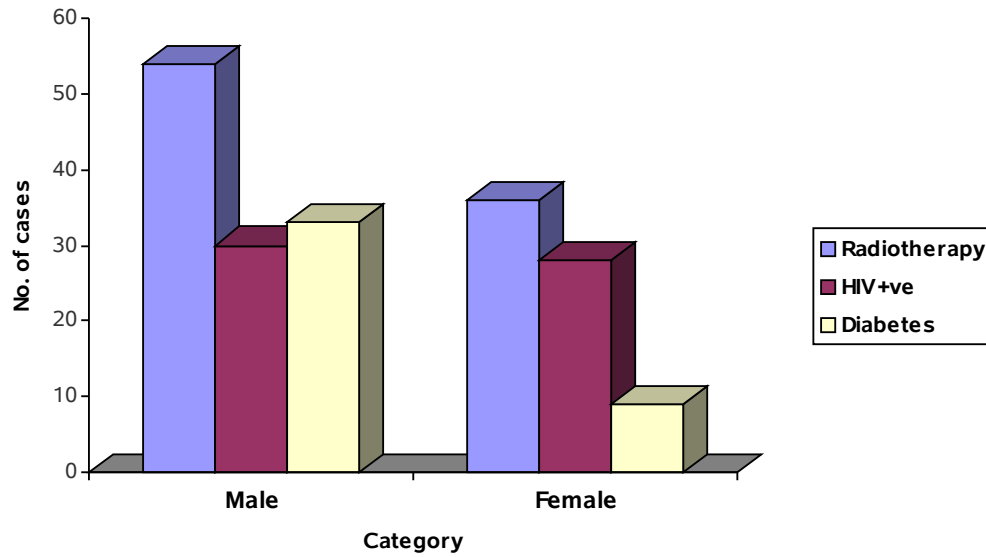


Fig2.Demographic character of the study population

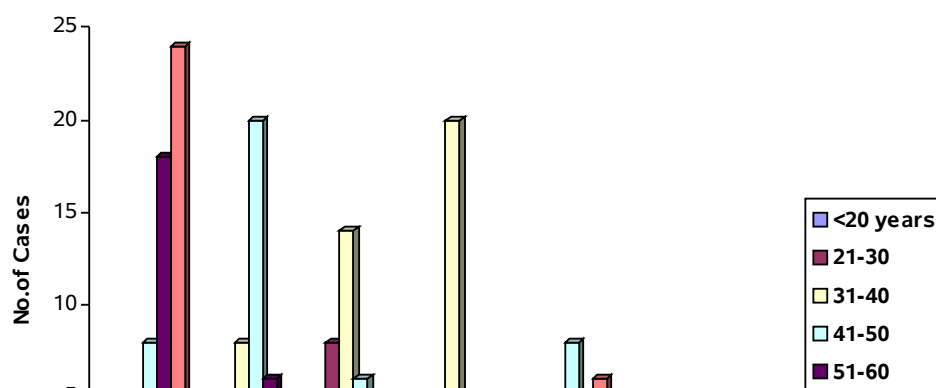


Fig2.Demographic character of the study population

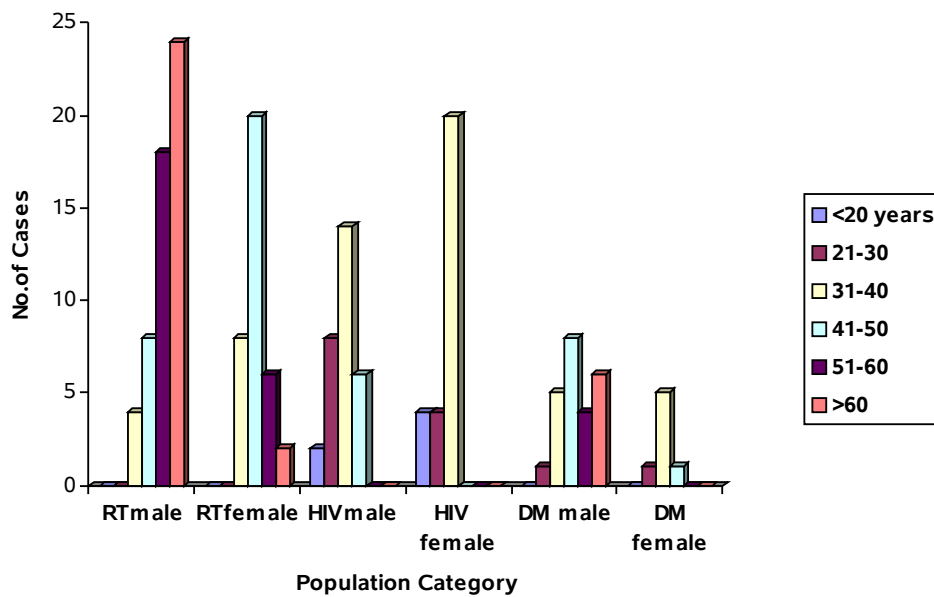


Table 3: Isolation of Candida in Immunocompromised individuals

Category	Total No. of samples	Directs smear & Growth on SDA		
		Male	Female	Total
Radiotherapy	90	42(65.6)	22 (34.4)	64 (71.1)
HIV	58	10 (33.3)	20 (66.7)	30 (51.7)
DM	42	31(79.5)	8(20.5)	39 (92.8)
Total	190	83 (62.5)	50 (37.5)	133 (70)

(Parenthesis denotes percentage)

RAPID IDENTIFICATION OF CANDIDA ALBICANS

Germ tube test

To confirm the identity as *C.albicans* the germ tube test was performed for all the **133** *Candida* isolates. Of the **133** isolates **68** (51.1%) showed the presence of germ tube and **65** (48.9%) showed no evidence of germ tube.

Chlamydospore production on Cornmeal Tween 80 medium (CMA-T80):

As a further confirmatory test, the **133** *Candida* isolates were grown on Cornmeal agar Tween80 medium (CMA-T80) for the production of chlamydospores. **74** (55.6%) isolates produced chlamydospores and **59**(54.4%) showed no chlamydospores on CMA - T80.

Fig.3 & 3a. Isolation of Candida in immunocompromised individuals

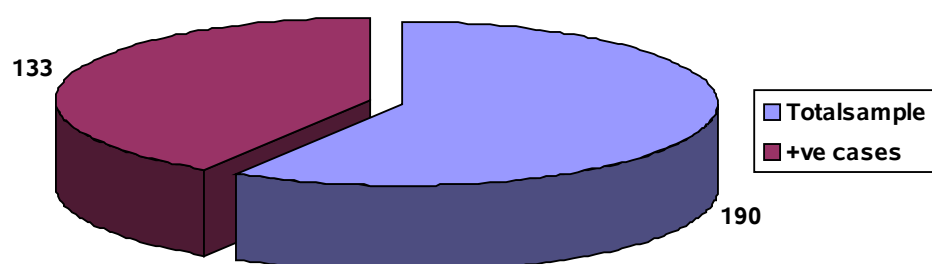


Fig.3a.

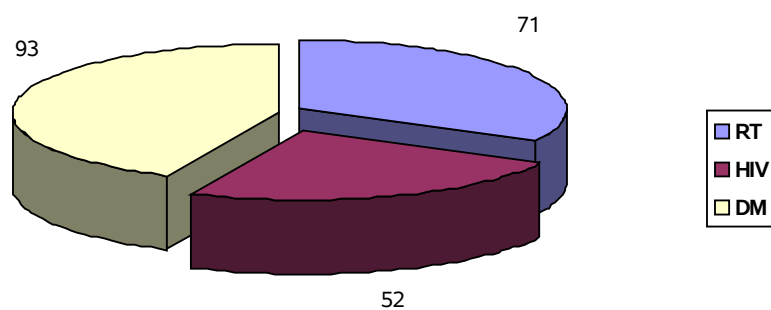
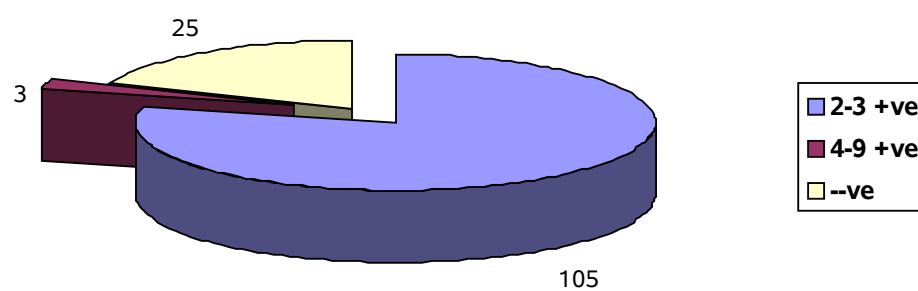


Fig.4. Lipolytic activities of Candida on Tween-80 Opacity medium



Chlamydospore production on Sunflower Seed Husk Agar

The **133** isolates from the SDA were cultured on the Sunflower Seed Husk Agar by furrowing technique and observed for the production of chlamydospores. Only **4** (3%) isolates produced rough colonies with hyphal fringes and abundant chlamydospores, **2** of which were confirmed as *C.dubliniensis* by further tests. The remaining **129** (97%) isolates produced smooth colonies with no evidence of chlamydospore production.

Tween 80 opacity test

In the Tween 80 opacity test, the **133** *Candida* isolates from the SDA were cultured (swabbing technique) and observed for the development of opalescence around the inoculation site due to the activity of the lipolytic enzymes, daily for 10 days.

Of the **133** isolates, **105** (78.9%) developed opalescence **within 3 days suggestive of the species *C.albicans* or *C.tropicalis***. Of this, **72**(54.1%) isolates were identified as *C.albicans*, as they showed the presence of germ tube on incubation with pooled human serum and production of chlamydospores on Corn meal agar with Tween 80 (CMA-T80) medium. **33**(24.8%) isolates were identified as *C.tropicalis* by the absence of germ tube and their morphology on CMA-T80 medium.

3 (2.25%) isolates developed **opalescence within 3-9 days and were identified as *C.lusitaniae*** by carbohydrate assimilation test.

25 (18.8%) isolates **did not develop opalescence even after 10 days** of incubation. Of this 2(1.5%) isolates were identified as *C.dubliniensis* by the production of germ tube, and chlamydospores on CMA T 80 & SSHA medium. (Table 4 &5)(Fig.4&5).

Table 4: Lipolytic activities of Candida on Tween 80 Opacity medium.

Total No. of isolates tested	No. of Isolates +ve			No. of Isolates - ve
	2 – 3 days	4 – 9 days	Total	10 – 12 days
133	105	3	108	25

Table 5: Lipolytic activities of various Candida spp. on a Tween 80 Opacity medium.

Species	Total No. of isolates tested	No. of Isolates +ve			No. of Isolates - ve
		2-3 days	4-9 days	Total	10-12 days
C.albicans	72	72		72	-
C. tropicalis	33	33		33	-
C.parapsilosis	7	-	-	-	7
C. kefyr	7	-	-	-	7
C. glabrata	6	-	-	-	6
C.guilliermondii	3	-	3	3	-
C.kusei	2	-	-	-	2
C.dubliniensis	2	-	-	-	2
C.lusitaniae	1	-	-	-	1
Total	133	105	3	108	25

Fig.5. Lipolytic activities of various Candida spp. on Tween-80 Opacity medium

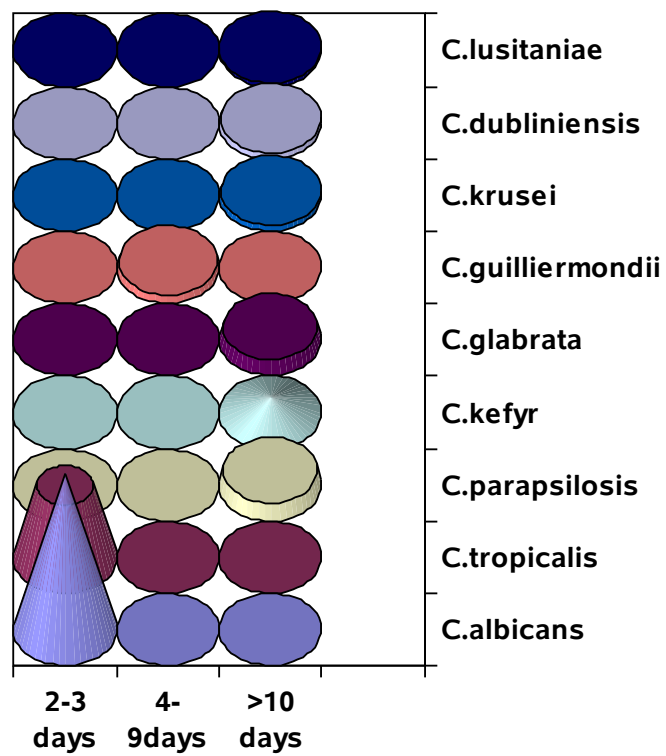
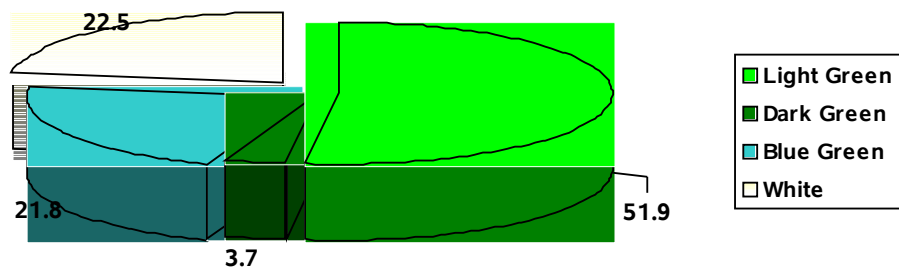


Fig 6. Isolation of Candida in CHROM agar Candida



CHROMagar Candida

The chromogenic substrate incorporated in the CHROMagar *Candida*, aided in the speciation of *Candida*, on the basis of specific colony colour particularly, for *C.albicans* (light green), *C.dubliniensis* (dark green), *C.tropicalis* (blue green hue) and *C.krusei* (light pink to white) and other species (white). Of the 133 isolates, **69** (51.9%) were light green, **5**(3.75%) dark green, **29** (21.8%) with blue green hue and **30** (22.55%) white in colour. (Table 6)(Fig.6)

Table 6: Isolation of Candida in CHROM agar Candida media

Colour	No. of positive Isolates	Species
Light Green	69 (51.9)	C.albicans
Dark Green	5 (3.7)	C. dubliniensis
Blue Green	29 (21.8)	C. tropicalis
White	30 (22.5)	Others
Total	133	

(Parenthesis denotes percentage)

Confirmative test for *C.albicans*

Among the 133 isolates, sensitivity of the Germ tube test was 51.2%; chlamydospores on CMA T-80 55.6%; absence of chlamydospores on SSHA 97%; Tween 80 Opacity test 81.3% and light green colonies on CHROM agar *Candida* 51.9%.

Table 7: Confirmative test for *C.albicans*

Name of the test	Positive		Negative	
	No	%	No	%
Germ tube Test	68	51.2	65	48.8
Chlamydospores +ve on CMA – T80	74	55.6	59	44.4
Chlamydospores +ve on SSHA	4	3	129	97
Tween-80 opacity Test	108	81.3	25	18.7
Light Green colonies on CHROM agar Candida	69	51.9	61	48.1

CMA - T80 : Cornmeal agar with Tween - 80

SSHA : Sunflower seed husk agar

Confirmation of *Candida* isolates

The speciation of the *Candida* isolates were confirmed finally by the **Carbohydrate assimilation test, morphology on Corn meal agar**, and the **Tween 80 opacity test** together with the other identification tests elaborated.

Morphology on Cornmeal agar Tween – 80

All the **133** *Candida* isolates from SDA were cultured on to cornmeal Tween – 80 agar for identifying the morphology and arrangement of the blastoconidia, chlamydoconidia, pseudohyphae and hyphal structures. **(Table 8) (Fig. 8)**

Fig.7 Confirmative tests for C.albicans.

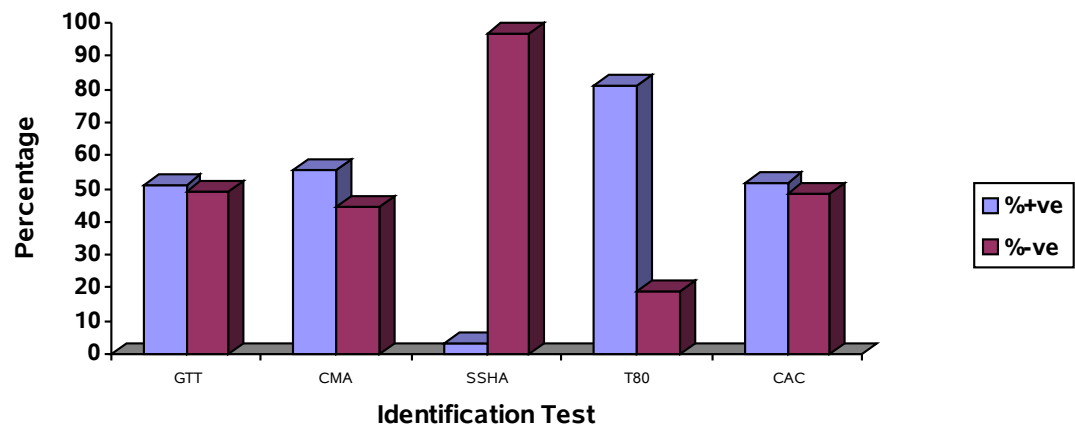
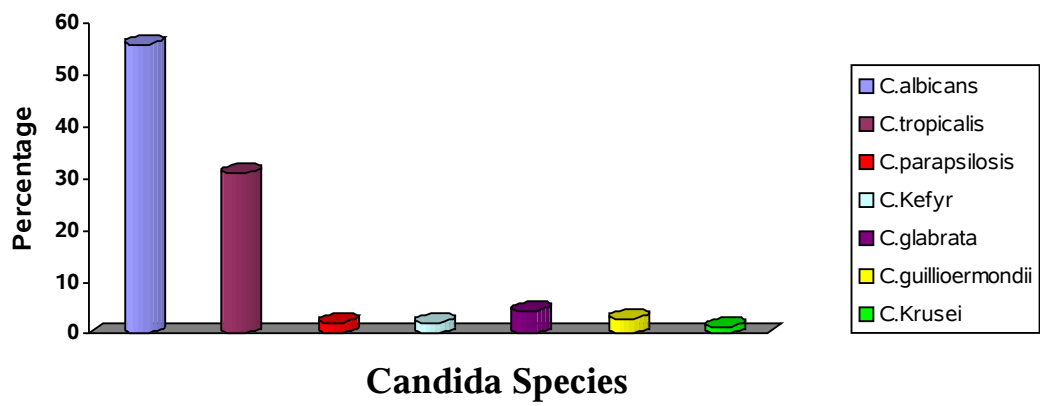


Fig.8. Candida species based on CMA T80 Morphology



Candida Species Assimilation Test

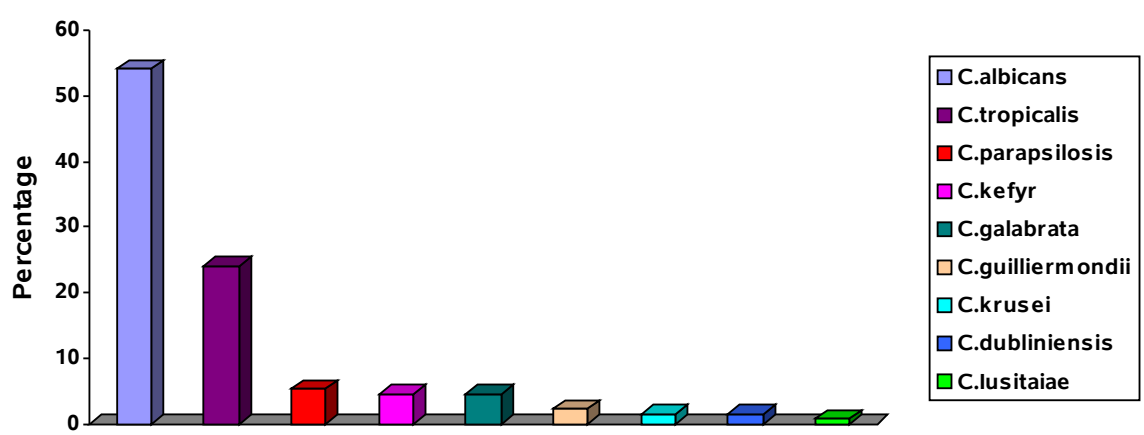


Table 8: Candida species based on blastoconidia, pseudohyphae and hyphal morphology

Morphology	Species	Total	Percentage
Compact clusters of blastoconidia (Intercalary or terminal) at regular intervals along pseudohyphae.	C.albicans	74	55.6
Abundant pseudomycelium composed of elongated blastoconidia widely spaced, in single or small clusters along the hyphae.	C. tropicalis	41	30.8
Thin, branching pseudomycelium with of few ovoid to elongate blastoconidia in verticils. Thick pseudomycelium and giant cells were also found.	C. parapsilosis	3	2.25
Abundant pseudomycelium with very elongate blastoconidia, fell apart and lie parallel like logs in a stream.	C. kefyr	3	2.25
No pseudomycelium. Small rounds yeast cells seen.	C. glabrata	6	4.5
Short pseudohyphae with clusters of small blastoconidia	C.guilliermondii	4	3
Elongated blastoconidia in tree or crossed match stick like appearance.	C.krusei	2	1.5
Total		133	

Carbohydrate Assimilation Test

Of the 133 *Candida* isolates 72(54.1%) were identified as *C.albicans*, 32 (24%) as *C.tropicalis*, 7 (5.3%) as *C.parapsilosis*, 6 (5.3%) as *C.kefyr*, 6 (4.5%) as *C.glabrata*, 3 (2.25%) as *C.guilliermondii*, 2 (1.5%) as *C.krusei*, 2(1.5%) as *C.dubliniensis*, 1 (0.75) as *C. lusitaniae*. (Table 9) (Fig.9)

Of the 133 isolates, 1(0.75%) isolate of *C.tropicalis* did not assimilate the sugar Galactose and 1(0.75%) isolate of *C.kefyr* did not. assimilate Xylose.

Table 9: Carbohydrate Assimilation Test

Species	Sucrose	Lactose	Galactose	Maltose	Xylose	Trehalose	Raffinose	Cellobiose	Mellibiose	Total
<i>C.albicans</i>	+	-	+	+	+	+	-	-	-	72 (54.1)
<i>C. tropicalis</i>	+	-	+	+	+	+	-	+	-	32 (24)
<i>C.parapsilosis</i>	+	-	+	+	+	+	-	-	-	7 (5.3)
<i>C. kefyr</i>	+	-	+	-	+	-	+	+	-	6 (4.5)
<i>C. glabrata</i>	-	-	-	-	-	+	-	-	-	6 (4.5)
<i>C.guilliermondii</i>	+	-	+	+	+	+	+	+	+	3 (2.2)
<i>C.krusei</i>	-	+	-	-	-	-	-	-	-	2 (1.5)
<i>C.dubliniensis</i>	+	-	+	+	-	±	-	-	-	2 (1.5)
<i>C.lusitaniae</i>	+	-	+	+	+	+	-	+	-	1 (0.75)
Total										133

(Parenthesis denotes percentage)

Distribution of Candida species among the clinical isolates

Together with all the identification tests the **133** isolates were speciated as **72** (54.1%) as *C.albicans*, **33** (24.8%) as *C.tropicalis*, **7** (5.3%) as *C.parapsilosis*, **7** (5.3%) as *C.kefyr*, **6** (4.5%) as *C.glabrata*, **3** (2.25%) as

C.guilliermondii, 2 (1.5%) as *C.krusei* , 2(1.5%) as *C.dublinsiensis*, 1 (0.75%) as *C. lusitaniae*.(Table:10)(Fig.10)

Table 10: Distribution of Candida species among the clinical isolates

Sl. No	Species	No. of Isolates	Percentage
1	C.albicans	72	54.1
2	C. tropicalis	33	24.8
3	C. parapsilosis	7	5.3
4	C. kefyr	7	5.3
5	C. glabrata	6	4.5
6	C. guilliermondii	3	2.2
7	C.krusei	2	1.5
8	C.dublinsiensis	2	1.5
9	C.lusitaniae	1	0.7
	Total	133	100

Identification of C.albicans – Comparison of test

In the idenification of C.albicans, the sensitivity for Germ tube test was 94.4%, Chromagar candida 95.8% and Sunflower Seed Husk Agar 97.2%. Whereas the sensitivity of chlamydospores on CMA – T 80, Tween 80 Opacity test & Carbohydrate assimilation test was 100%. (Table 11).

Fig. 10. Distribution of Candida species among the clinical isolates

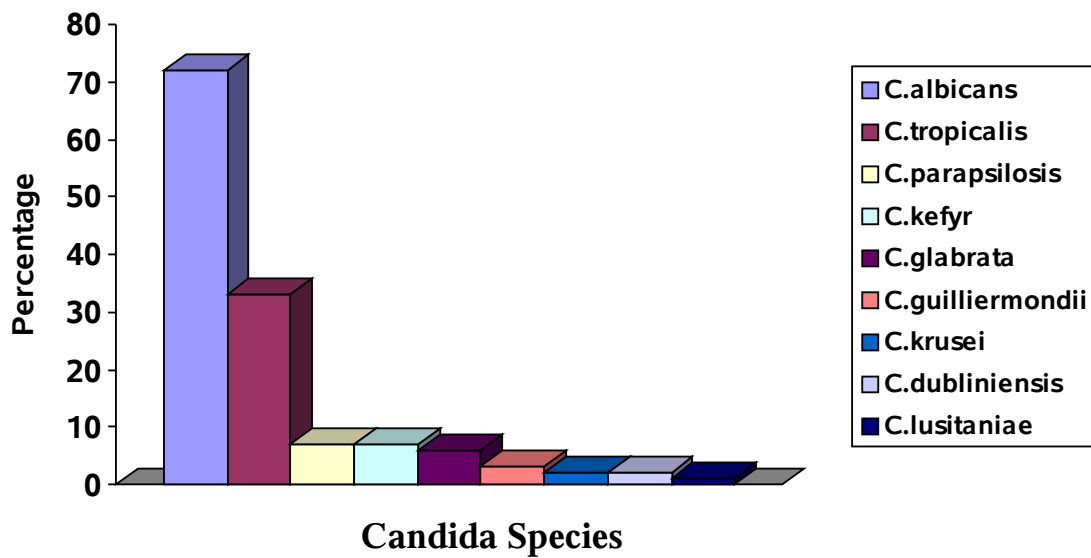


Fig. 12. Category wise distribution of Candida species

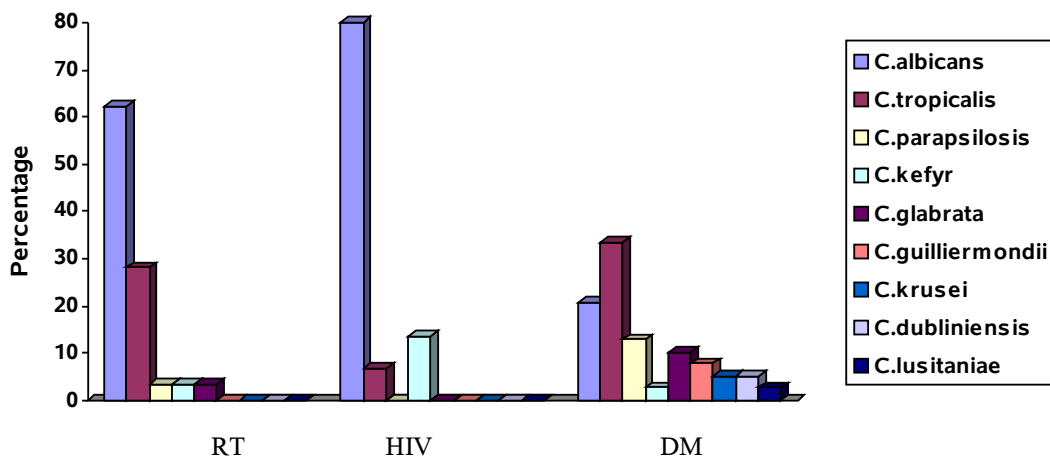


Table 11: Identification of Candida species – Comparison of tests

Identification test	No. of C.albicans isolates +ve	Percentage
Grams stain+ve	72	100

(budding yeast cell)		
Pseudohyphae +	54	75
Growth on S.D.A.	72	100
Germ tube Test	68	94.4
Chlamydospores +ve	72	100
Tween-80 opacity Test	72	100
Sun flower seed husk agar (Chlamydospores +ve)	70	97.2
C.A.C	69	95.8
Assimilation	72	100

Category wise distribution of Candida species

Among the 133 isolates, *C.albicans*-72 (54.1%) was the predominant species, followed by *C.tropicalis*-33(24.8%), *C.parapsilosis*-7(5.3%), and *C.kefyr*-7 (5.3%) *C.glabrata* -6 (4.5%), *C.guilliermondii* - 3 (2.25%), *C.krusei* -2 (1.5%), *C.dubliniensis*-2(1.5%) and *C. lusitaniae* 1 (0.75%).

In individuals who had been on radiotherapy *C.albicans* (62.5%) was the predominant species followed by *C.tropicalis* (28.1%).

In the HIV infected individuals also *C.albicans*(80%) was the predominant species followed by *C.tropicalis* (6.7%).

In the diabetics *C.tropicalis* (33.3%) was the predominant one followed by *C.albicans* (20.5%).

In the post radiotherapy patients non-*albicans* constituted 37.5%, in HIV infected individuals it was 19.9% and in diabetics it was 79.5% (Table: 12) (Fig.12)

Table 12: Category wise distribution of Candida species

Species	Department			Total
	R.T	HIV	DM	
C.albicans	40(62.5%)	24(80%)	8(20.5%)	72
C. tropicalis	18(28.1%)	2(6.7%)	13(33.3%)	33
C. parapsilosis	2(3.1%)	0	5(12.8%)	7
C. kefyr	2(3.1%)	4(13.3%)	1(2.6%)	7
C. glabrata	2(3.1%)	0	4(10.2%)	6
C. guilliermondii	0	0	3(7.7%)	3
C.krusei	0	0	2(5.1%)	2
C.dubliniensis	0	0	2(5.1%)	2
C.lusitaniae	0	0	1(2.6%)	1
Total	64	30	39	133

Sex wise distribution of Candida species

Isolation of *C.albicans* was almost equal among both sexes (51:49) whereas *C.tropicalis* was more in males. Non-*albicans* (61) were predominantly isolated from the male (72.1%) population than the females (27.9%). (Table 13) (Fig. 13)

Table 13: Sex wise distribution of Candida species

Species	Male	Female	Total
C.albicans	37(51.4)	35(48.6)	72
C. tropicalis	24(72.7)	9(27.3)	33
C. parapsilosis	6(85.7)	1(14.3)	7
C. kefyr	5(71.4)	2(28.6)	7
C. glabrata	3(50)	3(50)	6
C. guilliermondii	2(66.7)	1(33.3)	3
C.krusei	1(50)	1(50)	2
C.dubliniensis	2(100)	0	2
C.lusitaniae	1(100)	0	1
Total	81	52	133

(Parenthesis denotes percentage)

Age wise distribution of Candida species

The isolation of *C.albicans* was more in the age group 31-40years (33.3%), and that of non-*albicans* in the age group 51-60years (29.5%). The 2 *C.dubliniensis* were isolated from the age >60years (1.5%). (Table:14) (Fig.14)

Table 14: Age wise distribution of Candida species

Species	Below 20	21-30	31-40	41- 50	51- 60	Above 60	Total
<i>C.albicans</i>	6(8.3)	0	24(33.3)	14(19.4)	10(13.8)	18(25)	72
<i>C.tropicalis</i>	0	3(9.1)	4(12.1)	12(36.4)	9(27.3)	5(15.1)	33
<i>C.parapsilosis</i>	0	1(20)	2(40)	2(40)	2(28.5)	0	7
<i>C.kefyr</i>	0	0	4(57.1)	1(14.3)	2(28.5)	0	7
<i>C.glabrata</i>	0	0	1(16.7)	0	3(50)	2(33.3)	6
<i>C.guilliermondii</i>	0	1(33.3)	1(33.3)	0	1(33.3)	0	3
<i>C.krusei</i>	1(50)	0	0	0	1(50)	0	2
<i>C.dubliniensis</i>	0	0	0	0	0	2(100)	2
<i>C.lusitaniae</i>	0	0	1	0	0	0	1
Total	7 (5.3)	5 (3.7)	37 (27.8)	29 (21.8)	28 (21)	27 (20.3)	133

(Parenthesis denotes percentage)

Anti fungal susceptibility test

Of the **72** *C.albicans*, **61** (84.7%) were susceptible to Fluconazole, **70** (97.2%) to Itraconazole, **69**(95.8%) to Ketoconazole, **71**(98.6%) to Clotrimazole, 69 (95.8%) to Nystatin, and **71** (98.6%) to Amphotericin B.

Of the **33** *C.tropicalis*, **28**(84.8%) were susceptible to Fluconazole, **31**(93.9%) to Itraconazole, **32**(97%) to Ketoconazole, **30** (90%) to Clotrimazole, **30**(90%) to Nystatin, **32**(96%) to Amphotericin B.

Among the 7 *C.parapsilosis*, 6(85.7%) were susceptible to Fluconazole, 6(85.7%) to Itraconazole, 6(85.7%) to Ketoconazole, 5(71.4%) to Clotrimazole, 5(71.4%) to Nystatin, and 7(100%) to Amphotericin B.

Of the 7, *C.kefyr* 5(71.4%) were susceptible to Fluconazole, 5(71.4%) to Itraconazole, 5(71.4%) to Ketoconazole, 5(71.4%) to Clotrimazole, 5(71.4%) to Nystatin, and 7(100%) to Amphotericin B.

Among the 6 *C.glabrata* 1(16.7%) were susceptible to Fluconazole, 6(100%) to Itraconazole, 1(16.7%) to Ketoconazole, 1(16.67%) to Clotrimazole, 3(50%) to Nystatin, and 6(100%) to Amphotericin B.

Of the 3 *C.guilliermondii*, all were 100% susceptible to Fluconazole, Itraconazole, Ketoconazole, Clotrimazole, Nystatin, and Amphotericin B.

Among the 2 *C.Krusei*, both were only susceptible to Amphotericin B Of the 2 *C.dubliniensis*, both were only susceptible to Amphotericin B. *C.lusitaniae* was susceptible to all except Fluconazole. **(Table: 15)**

The over all antifungal susceptibility for the Candida isolated was 78.19% for Fluconazole, 94.7% for Itraconazole, 89.4% for Ketoconazole, 87.2% for Clotrimazole, 87.2% for Nystatin and 96% for Amphotericin –B.

Of this, the susceptibility of *C.albicans* was 84.7% for Fluconazole; 97.2% for Itraconazole; 95.8% for Ketoconazole; 98.6% for Clotrimazole; 76.4% for Nystatin and 98.6% for Amphotericin –B.

The susceptibility of non-*albicans* was 70.5% for Fluconazole; 91% for Itraconazole; 82% for Ketoconazole; 73.5% for Clotrimazole; 69% for Nystatin and 90.3% for Amphotericin –B.

Table 15: Anti fungal susceptibility pattern of the Candida

Species	Total	Fluconazole	Itraconazole	Ketoconazole	Clotrimazole	Nystatin	AmphotericinB
	S	S	S	S	S	S	S
C.albicans	72	61 (84.7)	70 (97.2)	69 (95.8)	71 (98.6)	55 (76.4)	71 (98.6)
C. tropicalis	33	28 (84.8)	31 (93.9)	32 (97)	30 (91)	26 (78.8)	32 (97)
C.parapsilosis	7	6 (85.7)	6 (85.7)	6 (85.7)	5 (71.4)	5 (71.4)	7 (100)
C. kefyr	7	5 (71)	5 (71.4)	5 (71.4)	5 (71.4)	5 (71.4)	7
C. glabrata	6	1 (16.6)	6 (100)	1 (16.7)	1 (16.7)	2 (33.3)	6 (83.3)
C.guilliermondii	3	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)
C.krusei	2	0	2 (100)	0	0	0	2 (100)
C.dubliniensis	2	0	2 (100)	2 (100)	0	0	2 (100)
C.lusitaniae	1	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	133	104 (78.2)	126 (94.7)	119 (89.4)	116 (87.2)	97 (72.9)	128 (96.0)

(Parenthesis denotes percentage)

Category

Fig. 13. Sex wise distribution of Candida species

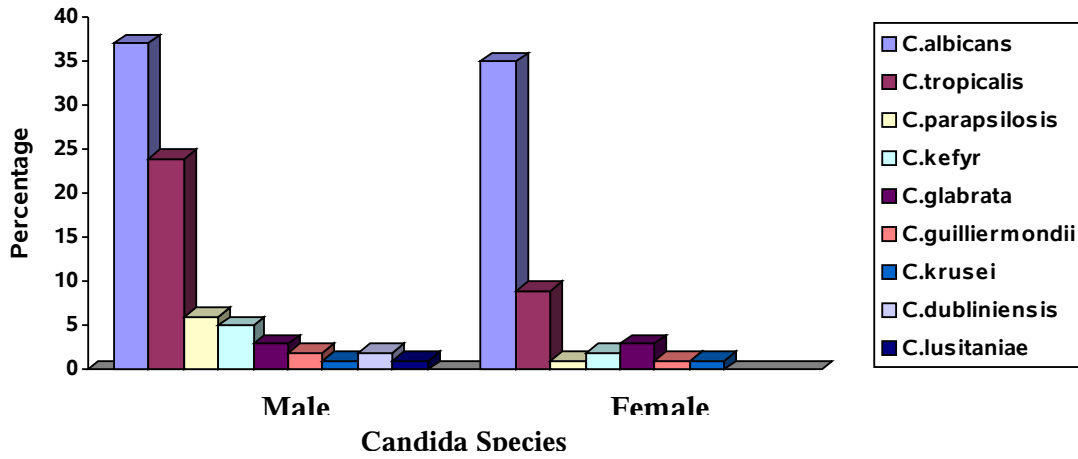


Fig.14. Age wise distribution of Candida Species

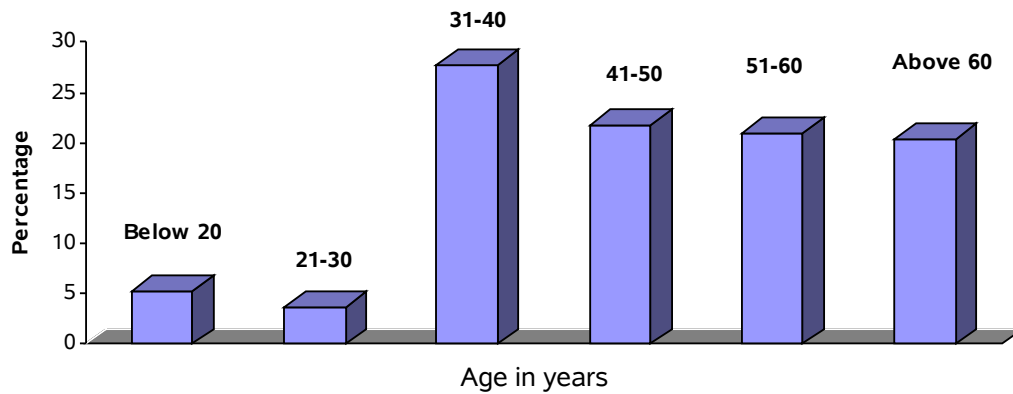
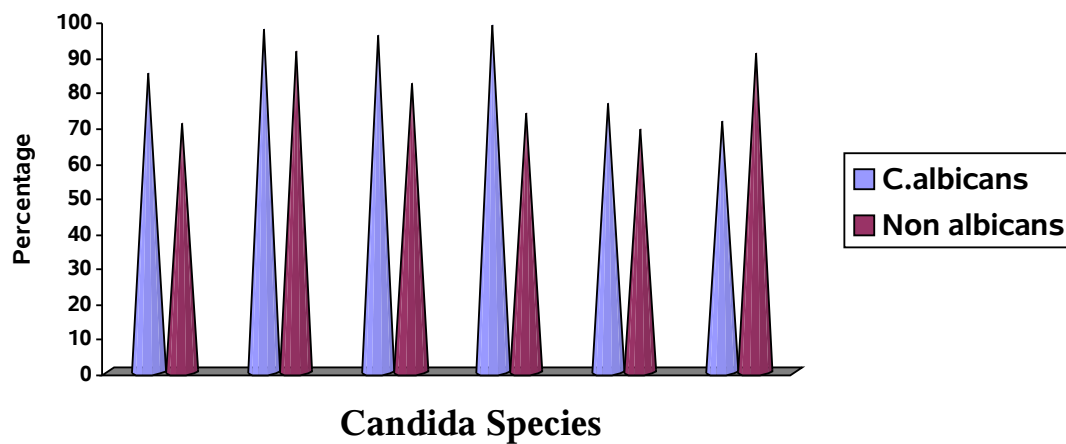


Fig. 15. Antifungal Susceptibility



DISCUSSION

The incidence of fungal infections has increased steadily over the past decade, due to the increase in *Candida* spp. other than *C.albicans*. Although *C.albicans* remains the most common species encountered as a cause of human infections, other *Candida* spp. have been increasingly associated with disseminated disease since the 1990. Since candidemia is associated with a high mortality rate, prompt appropriate antifungal therapy is essential in the immunocompromised.

A total of **190** clinical samples were collected from the Radiotherapy unit, Voluntary Counseling and Testing Center(VCTC), and the Diabetic Out patient Department of Tirunelveli Medical College Hospital for a period of 18 months to study the prevalence of oropharyngeal candidiasis(OPC) in the immunocompromised, to speciate the isolates and to find their antifungal susceptibility patterns.

In the present study, among the **190** study population, the occurrence of oropharyngeal candidiasis (OPC) was **133**(70%), of which **64** (71.1%) were from the patients who had received radiotherapy for oropharyngeal malignancies, **30** (51.7%) from HIV infected individuals and **39** (92.8%) from diabetic patients.

In our study the incidence of oropharyngeal candidiasis (OPC) was more (71%) among the patients who had received radiotherapy compared to the study of **Spencer W Redding** et al.¹⁴⁹ & **Finlay**.et.al.1996.⁴³ which was about 17 to 29%. [Ramirez-Amador V](#) et.al.,¹¹⁴ also studied that radiotherapy encourages oral *Candidal* colonization and 17.4% developed clinical candidiasis during radiotherapy. The present study correlates to the study of [Spijkervet FK](#) et al.,¹⁵⁰ with an incidence of 64%.

In the present study, the incidence of oropharyngeal candidiasis (OPC) among the HIV infected individuals was 51.7%. This correlates with the study of **Chien–Ching Hung** et al.,²⁴ where >50% of the HIV-infected outpatients were colonized with *Candida* spp. and 12% developed symptomatic candidiasis.

In our study, among the HIV infected individuals 16(53.3%) had CD4 counts <200cells/mm³. This is supported by the study of **Feigal D W** et al.,⁴¹ who demonstrated that progressive cell mediated immunodeficiency, with CD4+ counts <200cells/mm³, is a risk factor for colonization with *Candida* spp. and the development of candidiasis. In particular OPC occurs in up to 90% of patients during the course of HIV infection.²² **Fichtenbaum C J, Koletar S** et al., studied that up to 90% of persons with advanced untreated HIV infection developed OPC, with 60% having at least 1 episode per year with frequent recurrences (50-60%).

In our study, the association of diabetes and oropharyngeal candidiasis was about **92.8%**, which is in favour of **Knight I et al.**,⁷² who demonstrated that diabetics are more susceptible to candidiasis with an incidence of 67%. This is also supported by **Vazquez J A** and **J D Sobel et al.**, **1995**,¹⁶¹ who observed OPC mostly in diabetics (71%) with poorly controlled blood sugar levels. This was also supported by **Anton Y Peleg**, **Thilak Weeraratna et al.**, & **Soysa N S**, **Samaranayake L P**, and **Ellepola A N B et al.**,⁸ who showed an incidence of 82%.

In this study, the incidence of oropharyngeal candidiasis (OPC) was **83(62.4%)** in male and **50(37.6%)** in female. The high incidence in male was associated with the habit of smoking (**94%**) and alcoholism (**78%**) in males. This is in favour of the study by **Epstein J B et al.**,³⁸ who showed smoking and alcoholism as risk factors for oral colonization by *Candida* during radiation therapy as 87%.

When the age factor was taken into consideration, the incidence of OPC was more in the age group **>60years** (37.5%) in patients who had received radiotherapy. But the incidence was more in the age group **31-40 years** in the HIV infected (73.3%) and the diabetics (35.9%). This is in accordance with **Benito Almirante & Dolrs Rodriguez et al.**,¹² a population based surveillance where the age specific incidence rate was highest in infants (38.8 cases/100,000

population) and in those aged > 65 years (12 cases/100,000 population) for blood stream *Candida* infections.

Grams Staining and Growth on SDA

In the present study among the **190** immunocompromised population, the occurrence of oropharyngeal candidiasis (OPC) was identified as **70%**(133), by direct examination (Grams stain) of the smears from the oropharyngeal lesions for the presence of budding yeast cells and by the characteristically large, butyrous colonies on Sabouraud's dextrose agar (SDA). In Grams staining **40.6%** (54) showed the presence of pseudohyphae.

Germ tube test

Of the 133 (70%) *Candida* isolated from SDA, 68(**51.1%**) showed the presence of germ tube on incubation with pooled human serum. In our study among the 72 isolates identified as *C.albicans*, 68 (**94.4%**) produced the germ tube. This is in accordance with study of **Salkin L**, and **Hurd NJ** et al.,¹⁴² in which about 95% of *C.albicans* produced germ tube and 5% showed no germ tube production.

Chlamydospore production on Cornmeal Tween 80 medium (CMA-T80)

When the 133(70%) isolates were cultured on CMA T80 medium, 74 (**55.6%**) showed the production of chlamydospores. Of this 72 were identified as *C.albicans* in our study, which is in accordance with **Murray P R**, et al., in **the Manual of Clinical Microbiology, 6th edition.**⁸⁹

Chlamydospore production on Sun Flower Seed Husk Agar (SSHA)

When the 133(70%) isolates from SDA were cultured on SSHA, 4(3%) isolates produced rough colonies with hyphal fringes and abundant chlamydospores, of which 2 were confirmed as *C.dubliniensis* by other tests. The remaining 129 (97%) produced smooth colonies with no evidence of chlamydospores and were confirmed by further tests as *C.albicans*. This parallels the study of **Khan ZU** et al.,⁷⁰ & **Pinjon E** et al.,¹⁰⁷ where all *C. dubliniensis* isolates produced rough colonies with hyphal fringes and abundant chlamydospores whereas 96.2% *C.albicans* isolates produced smooth colonies with no evidence of chlamydospore production.

Tween 80 opacity testing

In our study among the 133 *Candida* isolates, **105(78.94%)** isolates **developed opalescence** around the inoculated site **within 3 days** on the Tween 80 medium. Of the **105** isolates, **72(54.13%)** were *C.albicans* and **33(24.81%)** *C.tropicalis* as confirmed by the other tests. **No opalescence** was seen even after **10 days** for **25(18.79)** isolates which were identified as *C.dubliniensis*(2), *C.parapsilosis*(7), *C.kefyr*(7), *C.krusei*(2), *C.guilliermondii*(3), *C.lusitaniae*(1) and *C.glabrata* (6) by the other identification tests. This parallels with the study of **Rudek W**¹³² & **Malcolm Slifkin**⁸⁰ who observed that *C.albicans* and *C.tropicalis* produced opacity within 3 days in all the 15 isolates tested, *C.guilliermondii* (5) and *C.rugosa* (3) produced opacity in 3-9 days and

C.dubliniensis (16) and other *Candida* (52) did not produce opacity even after 10days.

CHROM agar *Candida* (CAC)

In our study when the 133 isolates were grown on CAC, **69**(51.9%) isolates produced light **green** color colonies, **5**(3.7%) **dark green** colonies, **29**(21.8%) **blue green** colonies and **30**(22.5%) **white** colonies

Of the 72 *C.albicans* confirmed by other tests, only 69(95.83%). produced light green color and the remaining 3(4.1%) isolates produced dark green color.

Of the 33 *C.tropicalis* confirmed by other tests, only 29(87.87%) produced blue green colonies and 3(9.09%) produced white colonies.

Of the 25 white colonies 7 *C.parapsilosis*, 7 *C.kefyr*, 2 *C.Krusei*, 3 *C.guilliermondii*, 1 *C.lusitaniae*, 6 *C.glabrata* were identified by the other confirmatory tests. This is in favour of the studies conducted by [Hospenthal DR](#) et al.,⁶⁶ & [Murray CK](#) et al.,⁹⁰ who showed similar results.

Morphology of blastoconidia, pseudohyphae and hyphae on CMA T80

When the 133 isolates were cultured on CMA T80 to observe the morphology (blastoconidia, pseudohyphae and hyphae), **74**(55.6%) showed compact clusters of small, round blastoconidia at regular intervals along the pseudohyphae and also thick walled chlamydospores either intercalary or

terminal, in favour of *C.albicans*. Of this 2(1.5 %) isolates showed terminal chlamydospores in bunches of doublets and triplets favouring the morphology of *C.dubliniensis*.

Abundant pseudomycelium composed of elongated, widely spaced blastoconidia in or small clusters along the hyphae were noticed in 41 (30.8%) isolates which is in favour of *C.tropicalis* according to **Rippon JW Medical mycology pg 517.**¹³⁹

Thin branching pseudomycelium with verticals of few ovoid blastoconidia, few thick pseudomycelium and giant cells were observed in 3(2.2%) isolates is in favour of *C.parapsilosis* according to **Rippon JW Medical mycology pg 517.**¹³⁹

Abundant pseudomycelium with very elongated cells lying apart in parallel like logs in a stream appearance was noticed in 3(2.2%) of the isolates favouring the identification as *C.kefyr* as described in **Koneman, Color Atlas and Diagnostic Microbiology 5th edition pg 1043.**³⁴

In 6(4.5%) of the isolates only small round yeast cells were noticed. There was no pseudomycelium observed favouring the species *C.glabrata* as described by **Rippon JW Medical mycology pg 517.**¹³⁹

Short pseudohyphae with clusters of small blastoconidia were seen in 4(3%) of the isolates in favour of *C. guilliermondii* as mentioned in Rippon. JW Medical mycology pg 517.¹³⁹

Elongated cells in treelike arrangement with elongated blastoconidia was noticed in 2(1.5%) of the isolates suggesting the species *C.krusei* according to Rippon. JW Medical mycology pg 517.¹³⁹

Of the 41(30.82%) isolates suspected as *C.tropicalis* 4 (3%) were confirmed as *C.parapsilosis* and 4(3%) as *C.kefyr* by the assimilation studies. Similarly of the 4(3%) isolates identified as *C.guilliermondii* 1(0.75%) was confirmed as *C.lusitaniae*.

Carbohydrate assimilation tests

In the present study the assimilation pattern of the yeast isolates were consistent with that of the standard assimilation pattern, as described in the Elmer Koneman, Color Atlas and Text Book of Diagnostic Microbiology, 5th edition, 1043³⁴ with few variations.

Of the 133 *Candida* isolates 72(54.13%) were identified as *C.albicans*, 32 (24.8%) as *C.tropicalis*, 7 (5.26%) as *C.parapsilosis*, 6 (4.5%) as *C.kefyr*, 6 (4.5%) as *C.glabrata*, 3 (2.2%) as *C.guilliermondii*, 2 (1.5%) as *C.krusei*, 2(1.5%) as *C.dubliniensis*, 1 (0.7%) as *C. lusitaniae*. 1(0.07%) isolate of *C.tropicalis* did not assimilate the sugar Galactose and 1(0.07%) isolate of *C.kefyr* did not assimilate Xylose.

Prevalence of the Candida species:

In the present study conducted at Tirunelveli Medical College Hospital, the prevalence of *C.albicans* (54.1%) was more among the immuno-compromised followed by *C.tropicalis* 24.8%, *C.parapsilosis* and *C.kefyr* each constituted 5.26%, *C.glabrata* 4.51%, *C.guilliermondii* 2.25%, *C. krusei* and *C.dubliniensis* each 1.5% and *C.lusitaniae* 0.75%. This is in accordance with the study of **Luis Octavio Sanchez- Vargas et.al.**⁷⁹ who showed an incidence of 63.7%

In this study, *C.albicans* 72(54.1%) and *C.tropicalis* 33(24.8%) constituted 84.2% of the total. This is favoured by **Rippon D.W., in Medical Mycology; Candidiasis and the pathogenic yeast, pg 536**¹⁴¹ as these two species together account for about 80% of the isolates from medical specimens.

In our study **non-*albicans*** constituted **61**(45.86%), of which ***C.tropicalis*** was **33** (24.8%). This is in favour of **Luis Octavio Sanchez-Vargas et al.**,⁷⁹ who in their study of oral candidiasis in HIV infected, isolated 66.7% of *Candida*, of which. 27% were non-*albicans* spp. *C.glabrata* (18.7%) was the most frequent non-*albicans* followed by *C.tropicalis* (5.9%), *C.parapsilosis* (1.1%).

Our observation was in contrast to the study of **Fidel P L, Vazquez J A** and **Sobel J D**⁴⁴ who reported that *C.glabrata* was the second most frequently isolated *Candida* spp. from clinical specimens after *C.albicans*.

In this study, *C.albicans* was the predominant species in patients who had received **radiotherapy** (62.5%) and in **HIV** infected individuals (80%).In the **diabetics** *C.tropicalis* (33.3%) was the most frequent species isolated. This was favoured by **Winegard JR , Merz W G and Saral R** et al.,¹⁶⁹ observation of *C. tropicalis* (**40-80%**) as major pathogen in immunocompromised patients.

In our study, the prevalence of *C.albicans* was almost **equal** in both the sexes (51.4:48.6), where as in *C.tropicalis* it was **3:1**.

Antifungal susceptibility test

In our study **84.7%** *C.albicans* showed susceptibility for **Fluconazole** and > 95% for Itraconazole, Ketoconazole, and Amphotericin B.The overall susceptibility of non-albicans for Fluconazole is 70.5%. This is in favour of **Fichtenbaum CJ, Koletar S** et al.,⁴² where overall, the rates of Fluconazole susceptibility was 81%.

In our study, susceptibility to Clotrimazole was noted to be 87.2% and this is in favour of the study by **Pelletier, Joanne Peter** et al.,¹⁰² where the susceptibility was 70-96%. This is also supported by **Hood S, Evans J** et al.,⁶⁵ whose study showed a susceptibility of 50-63%.

In the present study, only 83.3% of *C.glabrata* and the 2 isolates of *C.krusei* were resistant to Fluconazole in favour of the study by **Pfaller** et al.,¹⁰⁴ where *C.glabrata* and *C.krusei* have been observed to be 4-fold to 32-fold less susceptible than *C.albicans* to Fluconazole.

The rates of ketoconazole and Itraconazole resistance in our study was 10.5% which was supported by **He X, Tiballi RN, Zarins LT**, et al.,⁶⁰ who reported 0% to 25% resistance in their study.

Similarly our study showed resistance to Amphotericin as 1.5% which correlates well with the study of **Ruhnke M, Eigler A**, et al.,¹³³ where the resistance was 1.3%.

In our study, *C.lusitaniae* was susceptible to Amphotericin – B in accordance with **Chien-Ching Hung, Yun-Liang Yang** et al.,²⁴ where the susceptibility was 100%. But our study was in contrast to the study of **Handfield** et al.,⁵⁹ where *C.lusitaniae* was reported to be relatively resistant to Amphotericin – B (0.4%) and Fluconazole (2.3%)

SUMMARY

This prospective study was conducted to evaluate the prevalence of *Candida* species among the immunocompromised individuals, mainly patients who had received radiotherapy for oropharyngeal malignancies, HIV infected individuals and diabetic patients.

The present study revealed that occurrence of oropharyngeal candidiasis was **more** in patients with uncontrolled **Diabetes mellitus** (92.8%) compared to 71.1% of the post radiotherapy patients and 52.7% of HIV infected individuals.

The incidence of oropharyngeal candidiasis was **more in males** (60.9%) attributed to the risk factors of smoking and alcoholism.

The overall incidence of oropharyngeal candidiasis was more in the age group 31-40 years (27.8%). The incidence was more in the age group **>60** years (37.5%) in the **radiotherapy** unit, and **31-40 years** (73.3%) in the **HIV** infected individuals and 41-50years (35.9%) in diabetic patients.

Among the immunocompromised individuals ***C.albicans*** was the predominant species in patients who had radiotherapy (62.5%) and in HIV infected individuals (80%); whereas ***C.tropicalis*** (33.33%) was the predominant species in the diabetics.

The prevalence of *C.albicans* was almost **equal** in both the sexes (51:49), where as that of *C.tropicalis* was **3:1**.

The incidence of *C.albicans* was high in the age group 31-40 years (33.3%) and that of **non-albicans** was in the age group **51-60** years (29.5%).

Of the total **190** OPC samples collected, **133**(70%) showed budding yeast cells on direct smear with Grams stain and growth on SDA

Of the 133 *Candida* isolates, 68(51.1%) showed the presence of the germ tube, 74(55.6%) showed the production of chlamydospores on Corn Meal Agar Tween80 (CMA-T80) medium, 4(3%) showed the production of chlamydospores on Sun flower Seed Husk Agar SSHA, 108(81.3%) produced opacity in Tween 80 medium. From this **72**(54.1%) were identified as *C.albicans* and 2(1.5%) as *C.dubliniensis*.

Speciation of the 133 isolates was done by observing the

- **Morphology on Cornmeal agar-Tween 80 medium**
- **Chlamydospores on Sunflower Seed Husk Agar medium**
- **Development of opacity on the Tween 80 medium**
- **Specific colour on CHROM agar *Candida* medium;**
- **Carbohydrate assimilation pattern on sugar free Yeast nitrogen base medium.**

Of the 133 isolates **72** (54.1%) were identified as *C.albicans* and **61**(45.8%) were **non-albicans**. Among the non-albicans 33(24.8%) were *C.tropicalis*; 7(5.7) *C.parapsilosis*; 7 (5.7) *C.kefyr*; 6 (4.5%) *C.glabrata*; 3

(2.2%) *C.guilliermondii*; 2(1.5%) *C.Krusei*; 2(1.5%) *C.dubliniensis*; 1 (0.75%) *C.lusitaniae*.

Of the 133 *Candida* isolates 78.2% were susceptible to Fluconazole; 94.7% to Itraconazole, 89.4% to Ketoconazole, 87.2% to Clotrimazole, 72.9% to Nystatin and 98% to Amphotericin –B.

Among *C.albicans* 84.7% were susceptible to Fluconazole and in non-*albicans* 70.5% were susceptible to Fluconazole.

CONCLUSION

As *C. albicans* is a normal human commensal, it is not surprising that this organism is the most common opportunistic fungal pathogen in immunocompromised patients.¹⁰⁵ Colonization of the oropharynx and/or the alimentary tract often precedes invasive yeast infections.

The incidence of oropharyngeal candidiasis is **high** among the **diabetics**.

The **males** are **more** affected due to the associated risk factors like smoking, alcoholism and tobacco chewing.

The age group **above 30yrs** is at high risk of developing oropharyngeal candidiasis.

C.albicans is the **most frequent** species isolated from the post radiotherapy patients and HIV positive individuals. *C.tropicalis* is the predominant species in the **diabetics**.

Direct microscopy using Grams stain and primary isolation on Sabourauds dextrose agar is the most simple, economic and easy method for the isolation of the *Candida*.

Early identification of C.albicans is by,

- ❖ **Germ tube test**

- ❖ **Production of chlamydospores on the Corn meal Tween 80**

- ❖ **Absence of chlamydospores on Sunflower Seed Husk Agar medium**
- ❖ **Opalescence within 3 days on the Tween 80 opacity medium**
- ❖ **Specific light green colour colonies on the CHROMagar Candida medium.**

The confirmation of the *Candida* species is by the morphology on the semi starvation medium Corn Meal Agar with Tween 80 and the Carbohydrate Assimilation pattern on the carbohydrate free, Yeast nitrogen base medium.

The **resistance of *non-albicans*** (28.5%) to **Fluconazole is more** compared to that of *C.albicans* (15.3%)

The **resistance to Itraconazole** is **less** compared to Fluconazole, 2.8% for *C.albicans* and for *non- albicans* is 8.2%.

The resistance to Amphotericin B is 1.5%.

Early identification of the newly emerging spp. *C.dubliniensis* is by

Production of **chlamydospores** on Sun flower Seed Husk Agar

Absence of opalescence on the Tween 80 opacity medium

Specific **dark green colour** colonies on CHROMagar *Candida* medium.

In ability to assimilate **Xylose**.

SUGGESTION

The oral manifestations of oropharyngeal infection in immunocompromised patients present a particular challenge for both medical and dental professionals as accurate diagnosis and appropriate treatment may be difficult. Effective control of infection is achieved by the judicious use of topical and systemic agents.

Therefore the isolation and identification of *Candida* spp. from clinical specimens are very essential in deciding the antifungal of choice.

Strategies for antifungal prophylaxis in cancer patients receiving intensive cytotoxic chemotherapy and radiotherapy have been based on the principle of reducing fungal colonization in the oropharynx and alimentary tract in order to reduce systemic invasive fungal infections during the period of greatest risk due to profound neutropenia.

Strategies to prevent severe candidal infections should be effective and safe, should not be associated with high likelihood for development of resistance to antifungal agents. The best strategy should focus on identification of the target population (those at risk for developing severe candidiasis), implementing simple but effective control measures, and when it is needed, providing antifungal chemoprophylaxis.

The choice of antifungal agent for prophylaxis in this population remains controversial. However, azole compounds such as Clotrimazole,

Ketoconazole and Fluconazole and Amphotericin B appear to be more effective and better tolerated than Nystatin suspension. [Reents S](#), [Goodwin SD](#), [Singh V](#) et al.¹²⁵

There is increasing evidence that prophylactic systemic azole antifungal can effectively reduce overall oral fungal colonization levels and reduce the risk of oral candidiasis, with fluconazole being the agent of choice. **Oral Complications of Chemotherapy and Head/Neck Radiation -US National Cancer Research Institute, 2006.**⁹⁹

The key economic advantage of prophylaxis with fluconazole or a combination of fluconazole with oral polyenes results from the reduction of the expected cost of subsequent fungal infection among those who are most at risk. [Wakerly L](#) et al.¹⁶²

Systemic antifungal chemotherapy can be associated with toxicity, cost, and emergence of resistance. An alternative approach is to give pre-emptive antifungal therapy, which consists of limiting antifungal therapy only to those patients at risk for serious candidal infections and who also exhibit evidence of significant colonization with candida spp. at two or more sites. This strategy has been proposed in the setting of cancer chemotherapy¹⁶⁰ and surgery.

More recent data, suggest that in the setting of extreme immune debilitation, a change in epidemiology of candidiasis has occurred with a reduction in the rates of C.albicans in favour of the non albicans spp. in

particular *C.glabrata*, *C.krusei*, *C.parapsilosis* and *C.tropicalis*.¹⁶⁷ Whether these changes are a consequence of increased immuno suppression, the use of prophylactic antifungal treatments or absence of adequate infection control measures is uncertain.

In general, routine prophylaxis of mucosal candidiasis, particularly OPC, has been discouraged.⁶

It should also be kept in mind that the best therapy for OPC is not an antifungal agent, instead

- Prevention of mucosal breakdown
- Suppression of microbial colonization
- Control of viral reactivation

Up to 58% of health care workers can carry candida spp. on their hands¹⁵⁴ and transmission from staff to patients and from patient to patient has been documented in several studies. Strict hand washing remains the most simplest and effective means to prevent the acquisition of organisms by patients.^{2,47}

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